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MORPHOLOGICAL, ANATOMICAL, AND PHYSIOLOGICAL CHANGES IN THE DEVELOPING FRUIT OF THE VALENCIA ORANGE, *CITRUS SINENSIS* (L.) OSBECK

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[Manuscript received August 5, 1957]

Summary

Measurements of fruit radius and peel and pulp width, as well as determinations of fresh weight, dry weight, moisture content, total and protein nitrogen content, and respiration rate were made throughout two growing seasons on Valencia oranges from the Gosford district of New South Wales. Soluble solids, sugar, and acid were also determined in the juice. Anatomical changes during development were investigated throughout one season.

Development could be divided into three stages, corresponding with changes in growth rate and coinciding on a calendar basis in both seasons. Stage I varied in length according to the date of the blossom, but was completed by mid December. This was the cell division stage; by mid December cell division was completed in all tissues except the outermost cell layers. Increase in fruit size at this stage was mainly due to increased peel thickness. Stage II, a period of very rapid growth from mid December to mid July, was the critical period for growth and was distinguished as the cell enlargement period, rapid morphological and physiological changes occurring in the absence of cell division. The growth of the pulp was responsible for most of the increase in fruit size during Stage II; the peel reached a maximum width early in this stage and then became thinner with very little subsequent change in thickness as the pulp continued to increase in size. Stage III, the maturation period, lasted from mid July until the fruit was ripe, or approximately 7 months. Fruit continued to grow for as long as it was left on the tree but at a very reduced rate compared with Stage II. Ripening occurred during Stage III.

I. INTRODUCTION

Citrus fruits develop slowly, the Valencia orange taking from 12 to 14 months to reach maturity. The tree fruits each year, blossoming again before the current crop is harvested.

Various aspects of development in several citrus fruits have been studied during part of the growing season and factors affecting quality in the mature fruit have received much attention (Harvey and Rygg 1936*a*, 1936*b*; Harding, Winston, and Fisher 1940; Sinclair and Ramsay 1944; Harding and Fisher 1945; Harding and Sunday 1949, 1953; Samisch and Cohen 1949; Marloth 1950; Rygg and Getty 1955). The work reported here, however, seems to be the first attempt to correlate changes in the morphology, anatomy, and composition of the fruit during its whole development on the tree.

Data were collected for the 1954 and the 1955 seasons to establish the general trends as the fruit develops on the trees, but no attempt was made to correlate changes in climatic and cultural conditions with fruit growth.

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Initially it was hoped to base the physiological data collected for the developing fruit on cell size, as for the apple fruit (Bain and Robertson 1951; Robertson and Turner 1951; Martin and Lewis 1952), but the morphology of the orange, its anatomy, and the histology of its various tissues proved too complex to permit quantitative interpretation of cell size changes.

Anatomical study has dealt mainly with changes in the number, size, and shape of cells in the various tissues of the fruit as it enlarged throughout the season. The detailed anatomy of citrus tissue is recorded in publications by Dufrenoy (1929), Ford (1942), Scott and Baker (1947), Scott, Schroeder, and Turrell (1947), and Webber and Batchelor (1948).

II. MATERIALS AND METHODS

(a) Source of Material

The oranges used in the investigation were supplied by the late Mr. G. Linton, Somersby via Gosford, N.S.W., from 30-year old trees. The rootstocks were rough lemon, the soil was a light sand, and irrigation was available. Five 2-lb dressings of ammonium sulphate were applied to each tree during the year.

Full blossom for the 1954 season was approximately November 17, 1953 (about a month later than the usual date), commercial picking taking place during December 1954, approximately 56 weeks after blossom. The final experimental pick was January 4, 1955; 59 weeks after full blossom. Full blossom for the 1955 season was approximately October 18, 1954, commercial picking being in late December, 61 weeks after full blossom, and the experimental picks continuing until February 27, 1956; 71 weeks after blossoming. The crop was heavier in the 1954 than in the 1955 season.

(b) Sampling

Samples were taken from eight trees, in two adjacent rows of four, in a corner of the orchard block in the 1954 season, the results being given as the average of eight trees. The number of experimental trees was increased to 16 in the 1955 season, i.e. the eight trees used in the previous season and a block of eight trees in the centre of the orchard. As no significant differences were observed between the two blocks of eight trees, the results presented are the means from 16 trees, except that one tree was completely picked by early September and another by the end of the following January. The last sample of the season was taken from a single tree.

Sampling an orange tree presents problems since various characteristics of the fruit (e.g. size, skin thickness, soluble solids to acid ratio of the juice, and the percentage juice) differ with aspect, exposure, and height on the tree.

The experimental work at Somersby was begun on the 1954 season fruit to establish general trends in development. Since sampling was to continue throughout the year from blossom to maturity, it was not possible to set aside a definite section and position on the trees, and samples were taken at random over the lower part of the tree.

TABLE 1

DATE OF PICKING, WEEKS FROM FULL BLOSSOM, SIZE, AND DISTRIBUTION OF SAMPLES

| Pick | Date | Weeks from Blossom | No. of Trees | Number of Fruits | | | | |
|------------------|-------------|--------------------------|--------------------|----------------------|--------------------|--------------------|-----------------------|--------------------------|
| | | | | Anatomical Sample | Moisture Sample | Nitrogen Sample | Respiration Sample | Total Fruit Picked |
| (a) 1954 Season* | | | | | | | | |
| 1 | 15. xii. 53 | 4 | 8 | 20 | 24 | 424 | — | 560 |
| 2 | 6. i. 54 | 7 | 8 | 20 | 24 | 200 | — | 240 |
| 3 | 26. i. 54 | 10 | 8 | 20 | 16 | 144 | 20 | 200 |
| 4 | 22. ii. 54 | 14 | 8 | 20 | 16 | 80 | 15 | 120 |
| 5 | 23. iii. 54 | 18 | 8 | 20 | 16 | 40 | 24 | 96 |
| 6 | 20. iv. 54 | 22 | 8 | 20 | 16 | 40 | 24 | 96 |
| 7 | 17. v. 54 | 26 | 8 | 20 | 8 | 24 | 24 | 80 |
| 8 | 15. vi. 54 | 30 | 8 | 20 | 8 | 24 | 22 | 80 |
| 9 | 12. vii. 54 | 34 | 8 | 20 | 8 | 24 | 20 | 80 |
| 10 | 9. viii. 54 | 38 | 8 | 20 | 8 | 24 | 19 | 80 |
| 11 | 6. ix. 54 | 42 | 8 | 20 | 8 | 24 | 20 | 80 |
| 12 | 5. x. 54 | 46 | 8 | 20 | 8 | 24 | 20 | 80 |
| 13 | 1. xi. 54 | 50 | 8 | 24 | 8 | 24 | 16 | 80 |
| 14 | 29. xi. 54 | 54 | 8 | 24 | 8 | 24 | 16 | 80 |
| 15 | 4. i. 55 | 59 | 8 | 24 | 8 | 24 | 15 | 80 |
| (b) 1955 Season† | | | | | | | | |
| Blossom | 18. x. 54 | 0 | 16 | 20 | 20 | 250 | — | 320 |
| 1 | 1. xi. 54 | 2 | 16 | 48 | 80 | 672 | 320 | 1120 |
| 2 | 29. xi. 54 | 6 | 16 | 48 | 80 | 832 | 160 | 1120 |
| 3 | 20. xii. 54 | 9 | 16 | 48 | 16 | 224 | 80 | 368 |
| 4 | 17. i. 55 | 13 | 16 | 48 | 16 | 240 | 32 | 336 |
| 5 | 14. ii. 55 | 17 | 16 | 48 | 16 | 240 | 48 | 352 |
| 6 | 14. iii. 55 | 21 | 16 | 48 | 16 | 112 | 48 | 224 |
| 7 | 18. iv. 55 | 26 | 16 | 48 | 16 | 64 | 48 | 176 |
| 8 | 16. v. 55 | 30 | 16 | 48 | 16 | 128 | 48 | 240 |
| 9 | 14. vi. 55 | 34 | 16 | 48 | 16 | 128 | 40 | 232 |
| 10 | 11. vii. 55 | 38 | 16 | 48 | 16 | 96 | 32 | 192 |
| 11 | 8. viii. 55 | 42 | 16 | 48 | 16 | 96 | 32 | 192 |
| 12 | 5. ix. 55 | 46 | 15 | 45 | 15 | 90 | 30 | 180 |
| 13 | 4. x. 55 | 50 | 15 | 45 | 15 | 90 | 30 | 180 |
| 14 | 31. x. 55 | 54 | 15 | 45 | 15 | 90 | 30 | 180 |
| 15 | 28. xi. 55 | 58 | 15 | 45 | 15 | 90 | 30 | 180 |
| 16 | 10. i. 56 | 64 | 15 | 45 | 15 | 90 | 30 | 180 |
| 17 | 31. i. 56 | 67 | 14 | 42 | 14 | 84 | 28 | 168 |
| 18 | 27. ii. 56 | 71 | 1 | 12 | 3 | 8 | 7 | 40 |

*Commercial picking approx. 56 weeks from blossom.

†Commercial picking approx. 61 weeks from blossom.

At the earlier picking dates large numbers of small fruits were taken to provide enough material for analysis; the number per sample was later decreased to ensure a supply of fruit until the end of the season. The fruits from each pick were allocated as shown in Table 1. In 1954, the fruits from each tree were sorted in the orchard into samples for anatomical study, and for the determination of moisture content, total and protein nitrogen content, and respiration rate. In 1955, the fruits from each tree were kept separate, and their fresh weight determined after removal of the respiration sample. In the 1955 season, no significant difference was found in the average fresh weight per fruit in the whole pick and in the sample for anatomical study. The latter was therefore considered to be representative of the whole pick at each sampling in both seasons. Moisture, total solids, total and protein nitrogen contents, and respiration rates were determined on separate samples at each pick.

(c) *Observations Carried Out at Each Sampling*

(i) *Morphological Changes*

Fruit radius, width of peel, and width of pulp segments were all recorded at each sampling as the means of four measurements along two equatorial diameters at right angles to each other. The radius of the central axis was estimated by subtracting the combined width of the peel and the pulp from the fruit radius. Fruit, peel, and pulp plus axis volumes were calculated from these measurements assuming the orange to be spherical. The peel was taken as being of even thickness over the fruit although it is thickest at the stem end, thinner in the equatorial region, and thinnest at the styler end.

(ii) *Anatomical Changes*

Anatomical studies were made mainly on material, preserved in 70 per cent. alcohol, from the 1955 season. The alcoholic solution caused the formation, especially in young fruit, of hesperidin crystals which had to be removed by alcoholic potassium hydroxide before microscopic examination. Sections cut by hand and material macerated by Jeffery's method (Johansen 1940) were mounted in glycerine jelly either unstained or stained in gentian violet or ruthenium red (0.02 per cent.). Other sections 12μ thick were cut by microtome on material embedded in paraffin wax.

(iii) *Physiological Changes*

The following determinations were carried out, first only on the whole fruit, and then on the separated peel and pulp plus axis samples as the fruit increased in size.

(1) *Fresh Weight*.—The fresh weight of each fruit in the anatomical sample was determined at each pick in both seasons. Peel and pulp fresh weights in fruit of the anatomical sample were found indirectly. Average fresh weights of peel and pulp determined on the sample for determination of nitrogen were used to calculate these quantities for each fruit in the anatomical sample.

(2) *Moisture Content and Total Solids*.—These were determined by drying thin slices of tissue in aluminium cans for approximately 24 hr in an air-draught oven followed by 15 hr in a vacuum oven at 70°F. The values for peel and pulp were combined to give the total moisture and solids of the whole fruit.

(3) *Nitrogen*.—Total and protein nitrogen were determined on dried material. From May 1955 onwards, total nitrogen was also determined on juice extracted from an additional sample of fruit. Nitrogen was determined in the stem and stylar halves of the peel and the pulp in the 1954 season.

Total nitrogen was estimated on 0.5 g of dried material using a modified micro-Kjeldahl method, the distillation being carried out using a modified Parnas-Wagner apparatus (McKenzie and Wallace 1954). The estimated nitrogen content of the peel plus that of the pulp gave the nitrogen content of the whole fruit.

The nitrogen content of 5 ml of thawed juice was determined in the above apparatus using a modification of the method set out by Beer and Patterson (1939) for cider and apple juice. The nitrogen content of the juice was calculated assuming that 1 ml of juice weighed 1 g and that the juice contained 10 per cent. of solids throughout the season.

Protein nitrogen was estimated on 1.0 g of dried material after extraction with 75 per cent. alcohol (Turner 1949).

Soluble nitrogen was recorded as the difference between the estimates of total and protein nitrogen.

Preparation of dried material was carried out as follows. Peel and pulp tissue were separated from January onwards in both seasons. The material was cut into thin slices, care being taken not to lose the juice when cutting the pulp in the later samples, and dried as previously described. The calyx and seeds were removed. The dried material was stored in airtight jars and subsequently ground to a fine powder.

Juice samples were prepared by passing juice squeezed from the cut halves of fruits on a lemon squeezer through a fine mesh sieve and freezing it for later analysis.

(4) *Respiration*.—Respiration rate was measured at 20°C by the Pettenkofer method, usually with eight continuous readings over 4 days. Duplicate samples were taken from the eight trees in 1954. Four samples, two from each of the groups of eight trees, were taken in 1955.

(5) *Estimation of Maturity*.—Maturity was estimated by changes in fruit colour and by changes in the total soluble solids, sugar, and acid content of the juice. Total soluble solids were estimated by a Brix hydrometer calibrated at 20°C and the percentage sugar was determined with a refractometer. The acidity of the juice was estimated by titrating 20 ml of juice with 0.2N sodium hydroxide with phenolphthalein as indicator. The acid content was expressed as anhydrous citric acid.

III. RESULTS AND DISCUSSION

Data for the two seasons, plotted on the basis of time from blossom, showed the same morphological and physiological trends but there was a lag of 4 weeks in the changes occurring in the second season. This period corresponded to the difference in blossoming time in the two seasons. Good agreement in morphological and physiological changes was shown between the seasons when data were plotted on a calendar basis, time of blossoming apparently having little effect on subsequent fruit development. It was then possible to distinguish three distinct stages in fruit development (Fig. 1). These three stages—from blossom to mid December, mid

December to mid July, and mid July to maturity—were each characterized by distinct morphological, anatomical, and physiological changes. The results have been presented to show these changes in each of the three stages, giving data for the 1955 season since most of the anatomical work was done in that season. The location of the tissues referred to in the text is shown in Plate 1, Figure 1. The ovary was made up of 9 or 10 (sometimes 8, 11, or 12) carpels, together forming the ovary wall, a ring of loculi, and the central axis. The ovary wall became the peel of the fruit and the carpel loculi the segments of the pulp.

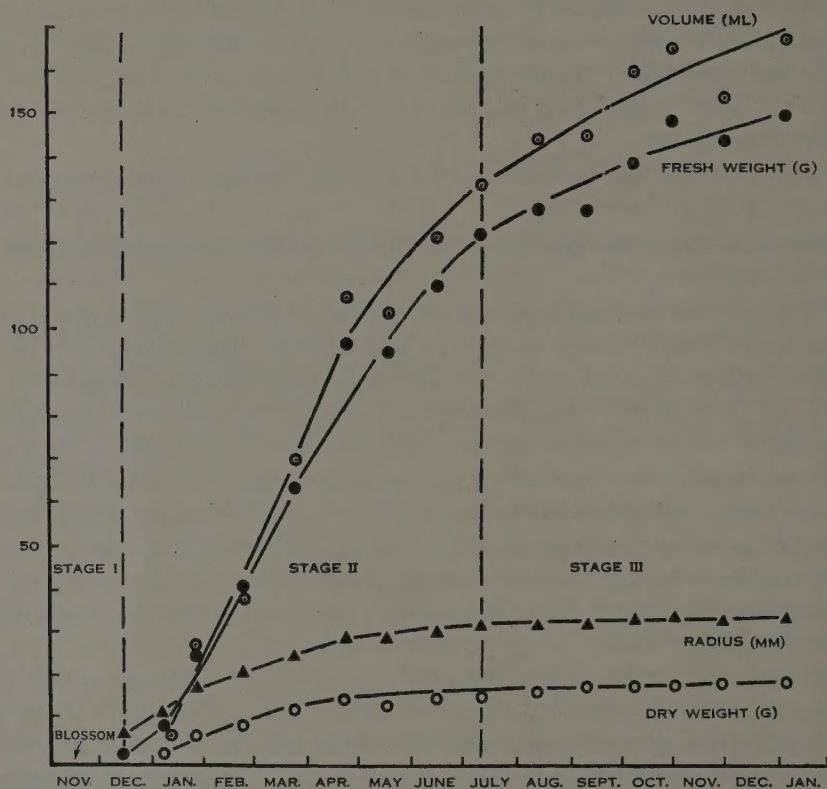


Fig. 1.—Fruit growth in the 1954 season, showing the three stages in development distinguished on a calendar basis.

The data are average values for fruits taken at each sampling and do not indicate the variation found between individual fruit nor do they indicate the considerable morphological and physiological variation known to exist within single fruits. Peel thickness decreased from the stem to the styler end of the mature fruit and was usually unevenly developed in a cross section of the fruit. Pulp segments frequently showed uneven increase in width on one side of the fruit. The existence of physiological differences between stem and styler halves of mature oranges is well established (Haas and Klotz 1935; Harvey and Rygg 1936a; Bartholomew and Sinclair 1941). Bartholomew and Sinclair (1941) reported differences in sugar

concentration and other soluble solids in adjacent pulp segments, and Hall (1955) found marked gradients in juice composition from the outer to the inner pulp in mature Valencia oranges. Total nitrogen determinations on peel and pulp at each sampling in the 1954 season showed consistent differences between the stem and stylar halves of the fruit throughout development. Total nitrogen per dry weight was significantly higher in the stem half of the pulp than in the stylar half ($P < 0.001$) and significantly higher in the stylar half of the peel than in the stem half ($P < 0.001$). Protein nitrogen per dry weight was not significantly different in the two halves during development.

(a) *Stage I. The Cell Division Period*

Stage I lasted from full blossom to mid December and was distinguished as the cell division period since the number of cells in all tissues of the developing fruit, excepting the inner cells of the central axis, increased to form the tissue of the mature fruit during that period. The peel of the fruit developed from the ovary wall, the tissue differentiating into the flavedo and albedo, and the juice sacs were formed in the pulp segments. Cell division was completed in all but the outer peel at the end of Stage I. Stage I lasted 4 weeks in the 1954 season and 9 weeks in the 1955 season. Data for the 1956 season showed that the longer period was more typical of Stage I. The 1954 fruit was smaller and had a lower fresh weight, dry weight, moisture content, nitrogen content, and respiration rate than the 1955 season fruit at the end of Stage I.

The morphological changes during the early part of fruit development are shown in Figure 2 and Plate 1, Figure 2.

Increase in fruit size during Stage I was mainly due to growth of the peel. The ovary wall made up one-third of the fruit in the blossom stage and the peel two-thirds of the fruit radius at the end of Stage I. The peel width was then about the same as the diameter of the pulp plus the central axis. Fruit volume increased from 0.04 ml to 3.7 ml during Stage I and the peel increased from 75 to 95 per cent. of the total volume.

The anatomical changes taking place at the same time as the morphological changes are shown in Table 2.

Cell division was active throughout the ovary wall of the blossom (Plate 2, Fig. 1). The increase in the volume of the tissues in the developing fruit was due to cell division followed by cell enlargement except in the outer peel where new cells formed continually. Growth of the peel in the first 2 weeks from blossom was due to increased cell division in the ovary wall. The volume of the outer peel continued to increase throughout Stage I by cell division; oil glands enlarged and new ones appeared (Plate 2, Figs. 2 and 3). Increase in volume in the rest of the peel was due to enlargement of the albedo cells and to increase in vascular tissue throughout the albedo; the vascular tissue was a network formed from a ring of bundles in the inner peel but did not have any connection with the developing pulp. Marked anatomical changes took place in the central albedo as the peel increased in thickness after cell division ceased. At this stage the tissue consisted of groups of recently divided cells and groups of cells with pectin-thickened walls (Plate 3, Fig. 1). These cells enlarged during the rest of Stage I and pectin became associated with all cells of the tissue (Plate 3, Figs. 2 and 3). The tissue was then very compact owing to the large amount

of pectin associated with each cell; the outer limit of the pectin layer around each cell was regular in outline but the inner limit was irregular. Plasmodesmata were seen in sectioned cells (Plate 3, Fig. 3). During the first 6 weeks from blossom, increase in the volume of pulp plus axis was mainly due to cell division in the septa between

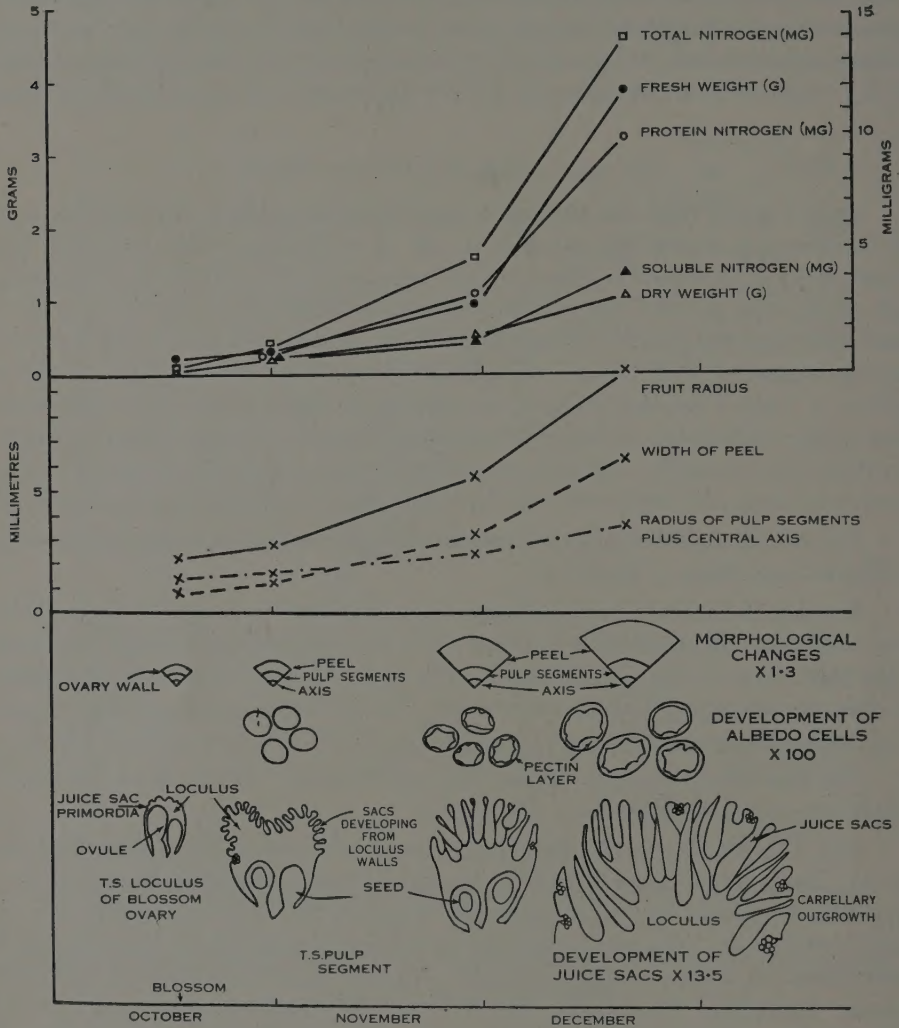


Fig. 2.—Developmental changes found in the fruit during Stage I from blossom to mid December.

the pulp segments and in the walls of the segments. Pulp segments increased in width at the end of Stage I by enlargement of the central cells of the septa and radial elongation of the marginal cells. Juice sac primordia, found in the blossom stage on the tangential and outer walls of the segments, continued to form during the next 2 weeks (Plate 3, Fig. 4). The sacs grew by cell division which continued at the head of the

TABLE 2
ANATOMICAL CHANGES OCCURRING DURING STAGE I IN FRUIT DEVELOPMENT

| Tissue | (Blossom) 18. x. 54 | 1. xi. 54 | 29. xi. 54 | 20. xii. 54 |
|---|---|--|---|---|
| <i>Ovary wall or peel</i> (a) Epidermis (b) Hypodermis (c) Flavedo (d) Albedo | Continuous cell division | | | |
| | Continuous cell division | | | |
| | 20-30 rows of undifferentiated cells; oil glands developing | 100 rows of cells—flavedo and albedo becoming distinct | Cell division continuing—oil glands enlarging and new ones forming | |
| | | | Albedo cells enlarging, especially in centre; pectin associated with cell walls | |
| | Vascular tissue increasing throughout albedo | | | |
| <i>Ovary loculi or pulp segments</i> (a) Septa (b) Juice sacs | 8-16 rows of cells across | 8-16 rows across | About 16 rows across; increasing radially by cell division | Cell enlargement; marginal cells elongating radially |
| | Primordia on tangential and outer radial walls of loculi | Number and size of primordia increasing by cell division | Cell division continuing in tips of sacs. Pulp segment one-third full | Cell division in tips; sacs stalked. Pulp segment half to two-thirds full |
| <i>Central axis</i> (a) Inner (b) Outer | 20-30 rows of cells across | Pectin becoming associated with cells—small air spaces | | Cells enlarging; air spaces and pectin increasing |
| | 12-15 rows of cells across | 20 rows | Approximately 50 rows | Cells becoming rounded; air spaces forming |

developing sac to form a club-shaped and then a stalked structure by the end of Stage I (Plate 3, Figs. 5 and 6). The enlarging juice sacs then filled one-half to two-thirds of the pulp segment. The outer part of the central axis grew in width by cell division followed by cell enlargement, the inner part by cell enlargement throughout.

Fresh weight, dry weight, moisture content, nitrogen content, and respiration rate changed rapidly in the developing fruit throughout Stage I (Figs. 2, 3, and 4). Protein nitrogen per fruit increased greatly during Stage I owing to the synthesis of new cytoplasm in dividing and enlarging cells but remained an unaltered fraction of the total nitrogen. Total and protein nitrogen decreased as a percentage of both fresh

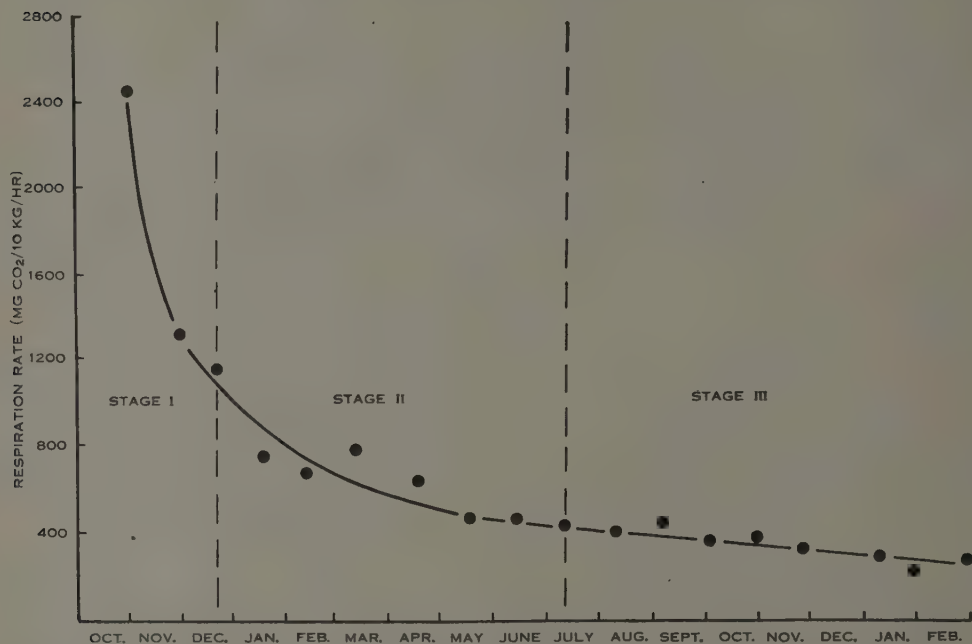


Fig. 3.—Respiration rate on a fresh weight basis throughout fruit development (measured at 20°C).

and dry weight. Total nitrogen fell from 2.6 to 1.3 per cent. and protein nitrogen from 1.8 to 0.9 per cent. per dry weight by the end of Stage I. This fall, reported previously by Cameron and Appleman (1934), corresponded to wall formation, increased vacuolation, and to the accumulation of solutes in the enlarging cells. Respiration rate per protein nitrogen dropped from 0.06 to 0.04 mg CO₂/hr during Stage I, possibly reflecting an increase in non-respiring protein in the cytoplasm.

(b) Stage II. The Cell Enlargement Stage

Stage II lasted for approximately 29 weeks from mid December to mid July and was the period of maximum fruit growth, morphological, anatomical, and physiological changes being very rapid throughout this period. During Stage II there was marked expansion of the tissues accompanied by cell enlargement, differentiation, and the formation of spongy tissue in the absence of cell division in all but the outer

peel tissues. The pulp expanded considerably; juice sacs became larger and juice content increased in their enlarging cells. The outer peel was yellow at the end of Stage II.

Stage II was the critical period for fruit growth and corresponded to the maximum growth period observed by Waynick (1927) in Californian Valencias during September to December. Waynick (1927, 1928) indicated that soil moisture, winds, and heat were important factors controlling growth during the critical period and that checks in growth during that time were not fully overcome in subsequent periods. The importance of this period of rapid growth was stressed by Marloth (1950) and Sauer (1953).

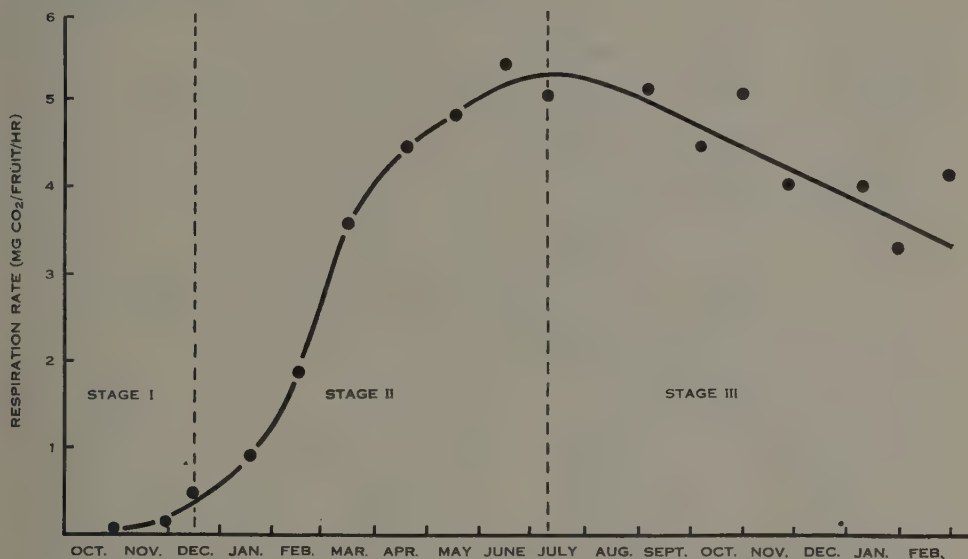


Fig. 4.—Respiration rate per fruit throughout development (measured at 20°C).

The changes in morphology of the fruit during Stage II are given in Figure 5. The size difference seen in the fruit of the two seasons at the end of Stage I disappeared by the end of the first 4 weeks of Stage II. During the last 4 weeks of Stage II in the 1955 season there was a marked check in the growth of the fruit.

Fruit radius increased threefold during Stage II, the increased size being mainly due to the growth of the pulp segments. Pulp plus axis volume increased more than 30 times to 67 ml during Stage II by radial expansion of the septa separating the segments and by enlargement of the juice sacs. Peel volume increased approximately 12 times to 49 ml, but decreased to 42 per cent. of the fruit volume during the same period. The peel reached its maximum thickness during the first 4 weeks of Stage II, became thinner in the next 14 weeks as if being stretched by the faster growth of the pulp, and changed little in thickness during the rest of Stage II. During Stage II there were marked anatomical changes in the peel, especially in the central albedo. These changes were necessary to counterbalance the rapid growth of the pulp in the absence of cell division in all but the outer peel tissues. Oil glands increased further in size throughout Stage II.

Anatomical changes accompanied the above morphological changes. These were especially marked in the central peel cells, in response to rapid pulp growth in the absence of cell division in all but the outer peel tissue. In the first 4 weeks of Stage II the peel grew in thickness, mainly by cell enlargement in the central albedo, and the irregularities of the pectin layer seen at the end of Stage I developed into

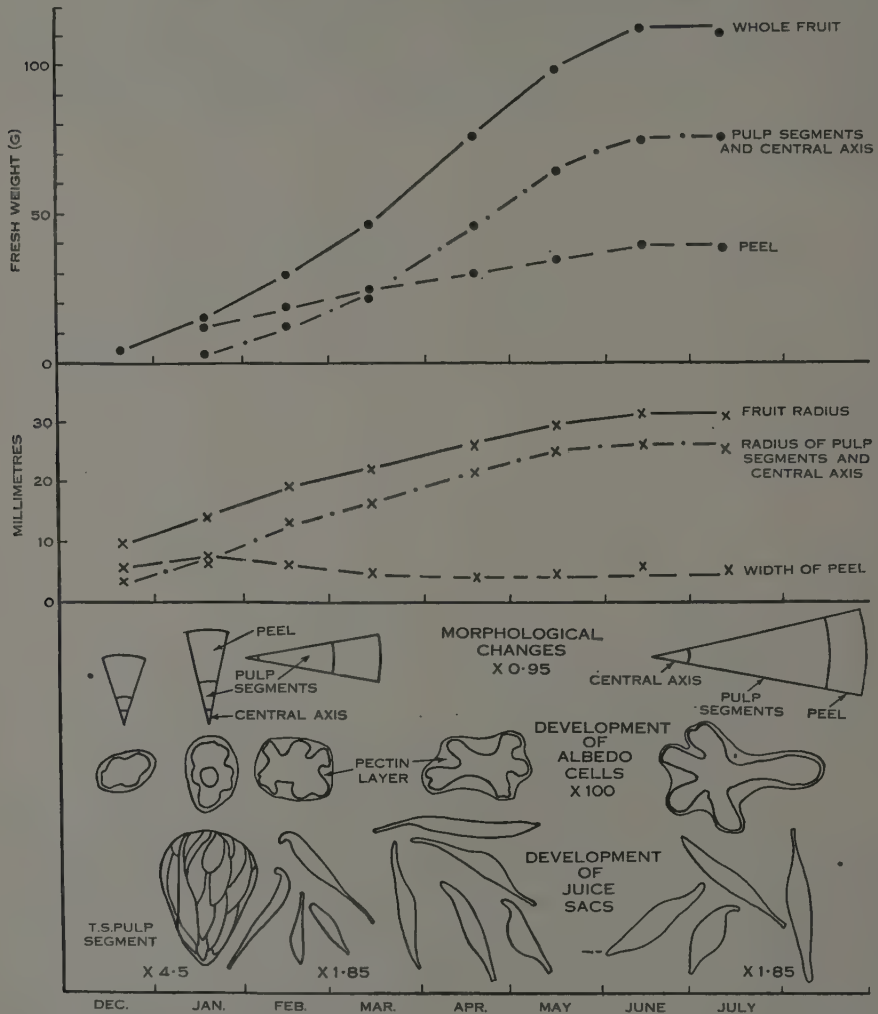


Fig. 5.—Developmental changes found in the fruit during Stage II from mid December to mid July.

small protuberances (Plate 4, Fig. 1). The tissue appeared compact in section, the pectin layer giving a regular outline to the cells. The accumulation of pectin in the cell walls of the peel is an important feature in the development of the fruit. Reed (1930) discusses the importance of hydrophilic colloids of cell walls in the transport of substances into the pulp of citrus fruits where there is no direct vascular contact

TABLE 3
ANATOMICAL CHANGES OCCURRING DURING STAGE II IN FRUIT DEVELOPMENT

| Tissue | 20. xii. 54 | 17. i. 55 | 14. ii. 55 | 14. iii. 55 | 18. iv. 55 | 16. v. 55 | 14. vi. 55 | 11. vii. 55 |
|----------------------|---|---|-----------------------------------|---|---|---|------------|-------------|
| <i>Peel</i> | | | | | | | | |
| (a) Epidermis | | | | | | | | |
| (b) Hypodermis | | | | | | | | |
| (c) Flavado | | | | | | | | |
| (d) Albedo | | | | | | | | |
| (i) Central | Cells enlarging; pectin associated with walls; little air space | Cells enlarging; spongy tissue forming | | Cells enlarging; further sponginess increasing | | Cells enlarging and stretching tangentially | | |
| (ii) Inner and Outer | | | | | | | | |
| | | Vascular tissue increasing throughout central peel tissue and branching towards the flavado | | | | | | |
| | | Cells enlarging and showing transitional stages in development of spongy tissue | | | | | | |
| <i>Pulp</i> | | | | | | | | |
| (a) Septa | Central cells enlarging; marginal cells elongating radially | Cells elongating radially; development of spongy central tissue begins | | Central cells distorted radially—sponginess of tissue increasing | | | | |
| (b) Juice sacs | Division continuing in tip of sac; some sacs stalked. Pulp segments one-half to two-thirds filled | Juice sacs enlarging—completely fill pulp segments after mid February | | | | | | |
| <i>Central Axis</i> | | | | | | | | |
| (a) Inner | Cells enlarging—air spaces and pectin increasing | Cells enlarging—air space increasing | Formation of spongy tissue begins | | Increase in sponginess; cells distorted | | | |
| (b) Outer | Cells becoming rounded—air spaces forming | Formation of spongy tissue begins | | Increase in sponginess—cells smaller, less distorted than in albedo | | | | |

between the peel and the developing juice sacs. The cells continued to enlarge in the next 4 weeks as the peel became thinner, their outgrowths increased in size, and air spaces developed in the tissue (Plate 4, Fig. 2). Pectin was thinner at the tips of the

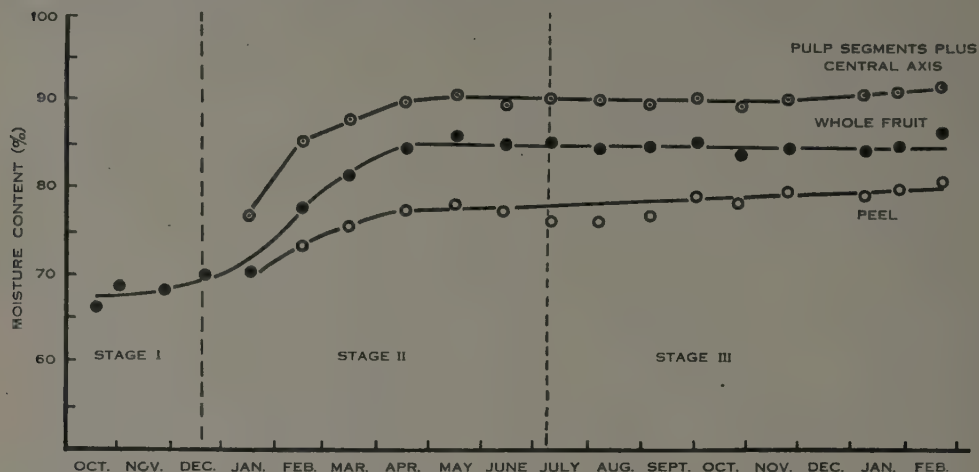


Fig. 6.—Percentage moisture content in the fruit, the peel, and the pulp throughout development.

outgrowths which represented the points of contact of the adjacent cells. Further peel thinning was accompanied by cell enlargement, the outgrowths lengthening to

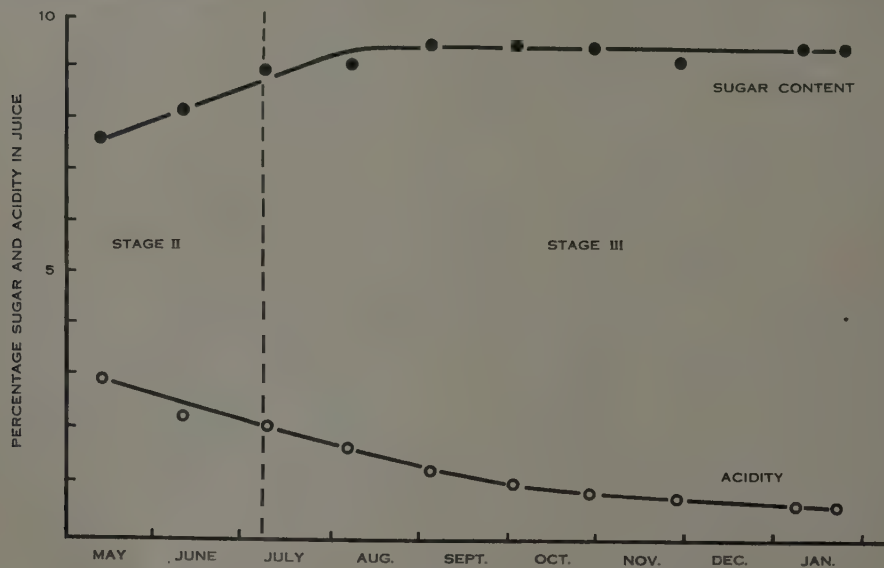


Fig. 7.—Percentage sugar and acid content in juice of maturing fruit.

form a spongy tissue made up of "spider-like" cells. The sponginess of the tissue increased still further in the later part of Stage II due to further cell enlargement and to tangential stretching of the central cells (Plate 4, Fig. 3). Transitional stages

in the formation of spongy tissue were found on either side of the central tissue. Elongation of the pulp septa was initially due to radial cell elongation; the cells then developed small protuberances and formed a spongy tissue in the first 8 weeks of Stage II. Juice sacs enlarged to fill the pulp segments, the central cells enlarging

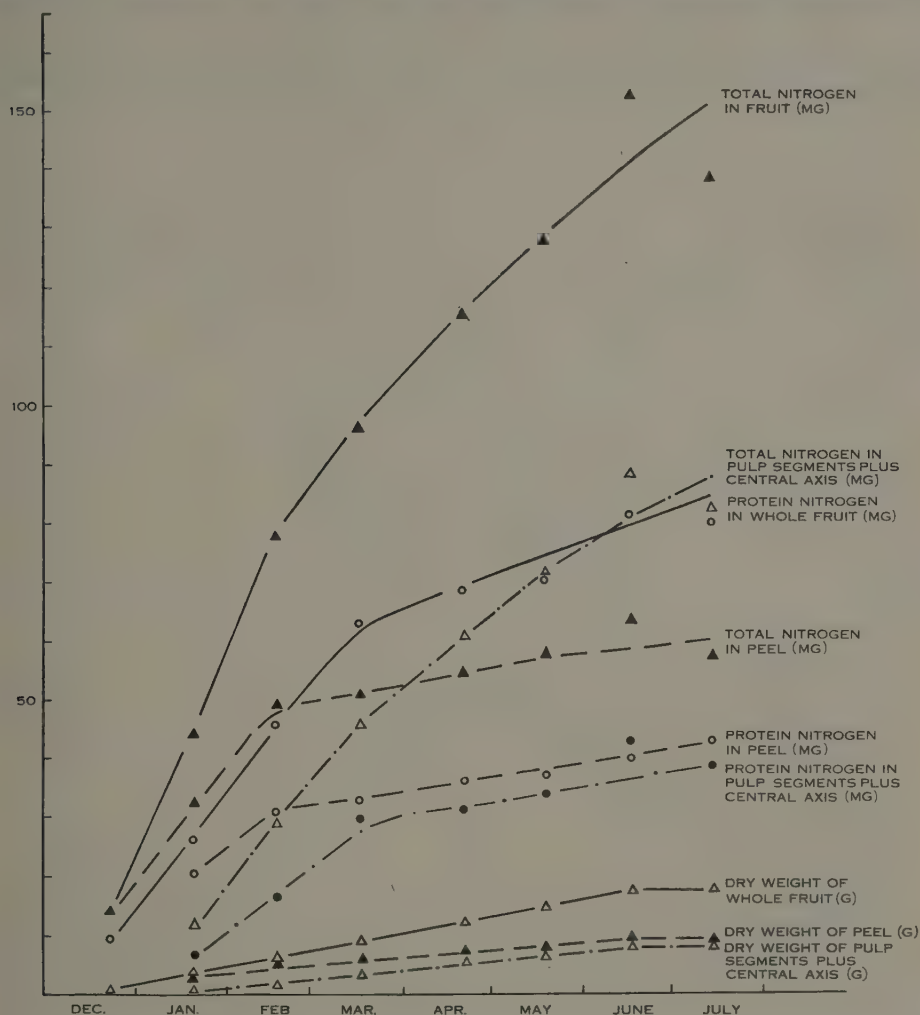


Fig. 8.—Changes in nitrogen content and dry weight during Stage II from mid December to mid July.

first. The central axis increased in size with the formation of spongy tissue, the inner cells becoming very distorted. The anatomical changes occurring during Stage II are set out in Table 3 and Figure 5.

Changes in fresh weight and dry weight during Stage II are shown in Figures 5 and 8. Increase in fresh weight in the whole fruit was mainly due to accumulation of water in the pulp segments, the juice sacs growing rapidly during this period. The

pulp plus axis increased from 20 to 67 per cent. of the total fresh weight and its moisture content from 21 to 71 per cent. of the total moisture in the fruit. The moisture content of the peel and of the pulp, expressed as a percentage fresh weight, increased markedly until April and then there was little change in this value during the rest of Stage II (Fig. 6). The concentration of the juice increased (the Brix reading rose from 8.3 to 8.9°) and the acidity decreased during the later part of Stage II (Fig. 7). The peel changed colour during the same time, turning light green during April, yellow by mid June and orange by the end of Stage II. Respiration

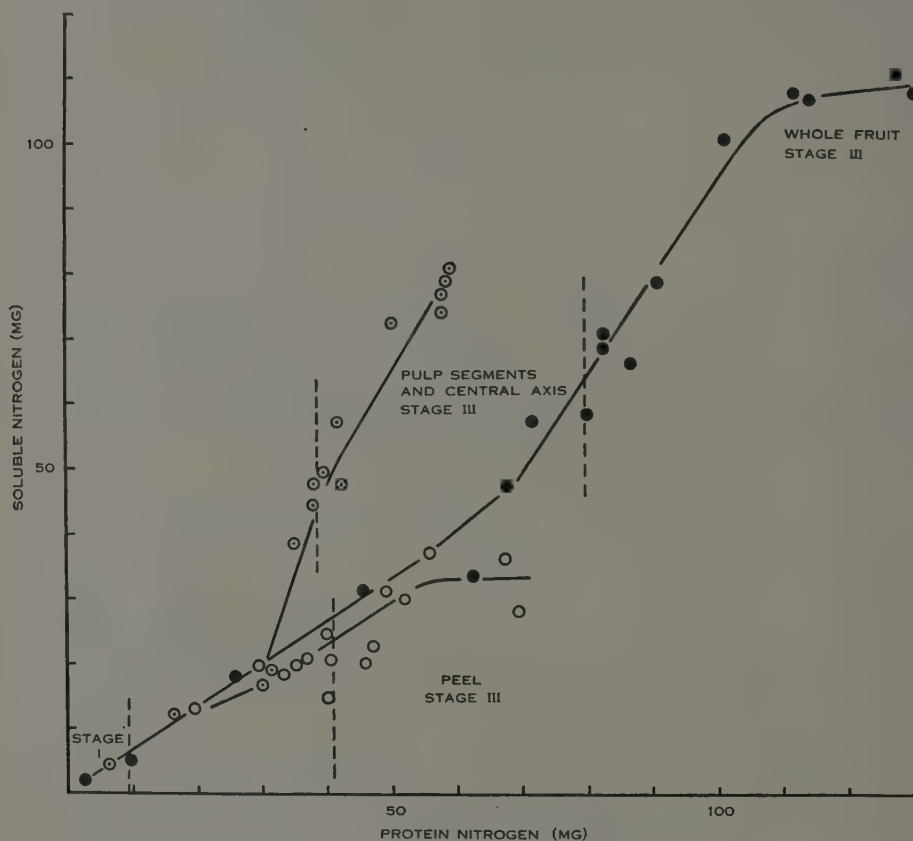


Fig. 9.—Changes in soluble and protein nitrogen content throughout fruit development.

rate per weight decreased to one-third though the rate per fruit increased by approximately ten times during Stage II (Figs. 3 and 4). Respiration rate per protein nitrogen reached a maximum of 0.07 mg CO₂/hr during April to June and began to decrease at the end of Stage II.

Changes in nitrogen level in the whole fruit, the peel, and the pulp segments plus the central axis are shown in Figure 8. Nitrogen increased rapidly in the fruit throughout Stage II, although the rate of increase slowed slightly after February. The peel contained most of the nitrogen in the fruit early in Stage II. Nitrogen

content of the peel increased considerably in the first 4 weeks of rapid cell enlargement; this was mainly due to increase in protein nitrogen; further increase in nitrogen of the peel after February was slow. Nitrogen accumulation in the pulp was rapid throughout Stage II, the pulp containing most of the nitrogen of the fruit after April. Most of the nitrogen in the pulp at the end of Stage II was in the juice (66 per cent.).

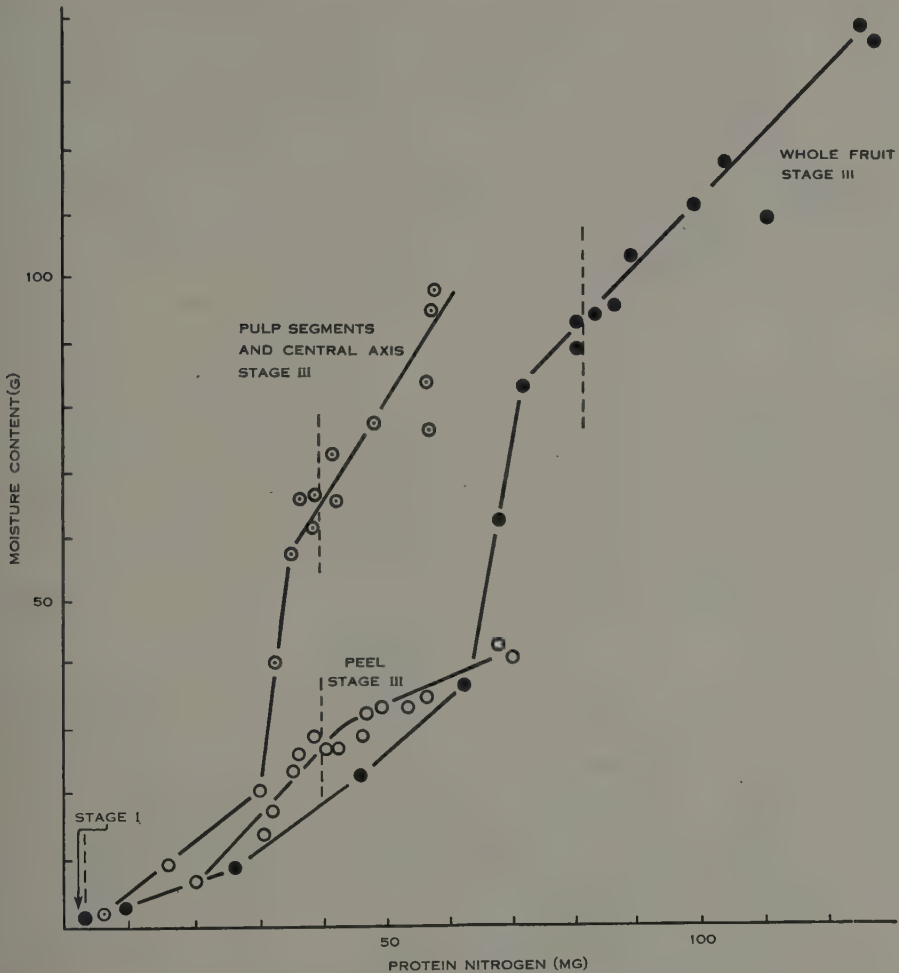


Fig. 10.—Changes in moisture content and protein nitrogen level throughout fruit development.

The percentage total nitrogen, expressed per dry weight, fell throughout Stage II in the whole fruit, the peel, and the pulp. Protein nitrogen made up the bulk of the nitrogen in the fruit throughout Stage II, but decreased from 70 to 58 per cent. of the total nitrogen during this period owing to increase in soluble nitrogen. Most of the protein nitrogen was in the peel in the early part of Stage II. Protein nitrogen increased rapidly in peel and pulp until February and March and this was presumably associated with formation of cytoplasm in enlarging cells; thereafter increase was

slower in both tissues, the peel maintaining a slightly higher amount than the pulp. The percentage protein nitrogen in the whole fruit, the peel, and the pulp fell throughout Stage II when expressed per dry weight.

Soluble nitrogen in the pulp increased rapidly after March with little change in protein nitrogen (Fig. 9). This rapid increase in soluble nitrogen accompanied the entry of juice into the juice sacs and showed that these cells were expanding in size without further protein synthesis. The moisture content of the pulp increased 3.3 times while the protein nitrogen content increased 1.3 times from mid March to mid June (Fig. 10). This sudden rise in moisture content with little change in protein nitrogen, following a period when moisture and protein increased together at a rate consistent with normal vacuolation, was even more marked in the 1954 season. Energy was apparently available for synthesis during the later half of Stage II as the respiration rate of the whole fruit during March to June (the rate for pulp was not measured separately) was then higher per protein nitrogen than at any other time, and the sugar content of juice increased.

(c) *Stage III. The Maturation Period*

Stage III, regarded as the maturation period, lasted from mid July until commercial maturity and later, and was readily distinguished from Stage II by decreased rates of morphological, anatomical, and physiological changes, by the flavedo changing in colour from yellow to orange, and by a decrease in titratable acid in the juice. Increase in fruit size, fresh weight, dry weight, moisture, and nitrogen content continued as long as the fruit was on the tree, the increases being mainly in the pulp plus central axis (Figs. 11 and 12).

Data were collected for 25 weeks (ending 3 weeks after commercial picking) in the 1954 season and for 33 weeks (ending 10 weeks after commercial picking) in the 1955 season; the fruit was considered small in both seasons. The cessation of growth found at the end of Stage II continued into the first 2 months of growth of Stage III in the 1955 season, the lack of growth being attributed to very wet conditions during that period. The 1955 fruit, however, recovered from the growth check to reach a size comparable with that of the heavier crop in the 1954 season.

Fruit radius increased by 5.0 mm during Stage III owing to increased size of the pulp segments, increased width of the central axis whose spongy tissue split and pulled apart, and to the growth of the peel. The peel increased little in thickness during this period but its tangential growth kept pace with the enlarging pulp. Peel volume increased at about the same rate as pulp volume. Marloth (1950) indicated that the peel of Valencias increased gradually between 5 to 16 months after fruit set, and that "when twice the rind thickness was expressed as a percentage of the total diameter of the fruit, it was found that (except for higher values while the fruit was small and immature) rind thickness remained directly proportional to fruit diameter".

The radius and the peel and pulp width of individual fruit varied considerably at each picking. Since peel thinning in the early part of Stage II was associated with increasing pulp development it was thought that at maturity the larger fruits might have a greater proportion of pulp and a thinner peel. Such a correlation, however,

did not exist when individual data for 96 fruits were examined. Mature fruit showed no correlation between peel thickness and fruit radius, e.g. fruits of radius 33.0 mm had peels which were 4.7 and 7.7 mm thick. Fruits of different radii had the same peel thickness, e.g. fruits of radius 29.5 and 36.8 mm had peels which were 5.0 mm thick. No correlation was found between peel thickness in mature fruits and the

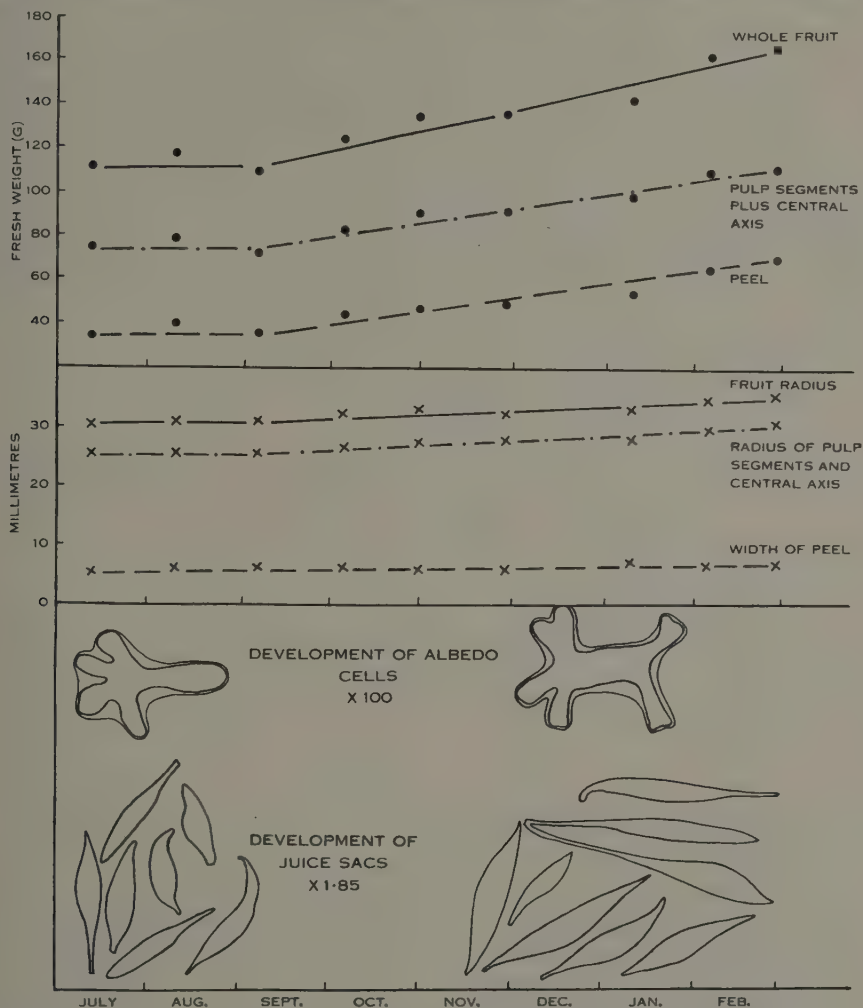


Fig. 11.—Developmental changes found in the fruit during Stage III from mid July to maturity.

number of pulp segments, which varied from eight to twelve. The thinnest sector of peel in a fruit was not always adjacent to the widest area of the pulp. It was, therefore, likely that average peel thickness in the individual mature fruit depended on the maximum thickness reached during Stage I or early Stage II and on the subsequent amount of thinning in the later part of Stage II after which the peel increased in volume with little alteration in thickness.

Continued cell division in the epidermis and hypodermis, growth of oil glands, modification of the flavedo cells, and further enlargement and distortion of the central albedo cells increased the volume of the peel (Plate 4, Fig. 4). The flavedo cells of the mature peel had thick deposits of pectin associated with their walls; the

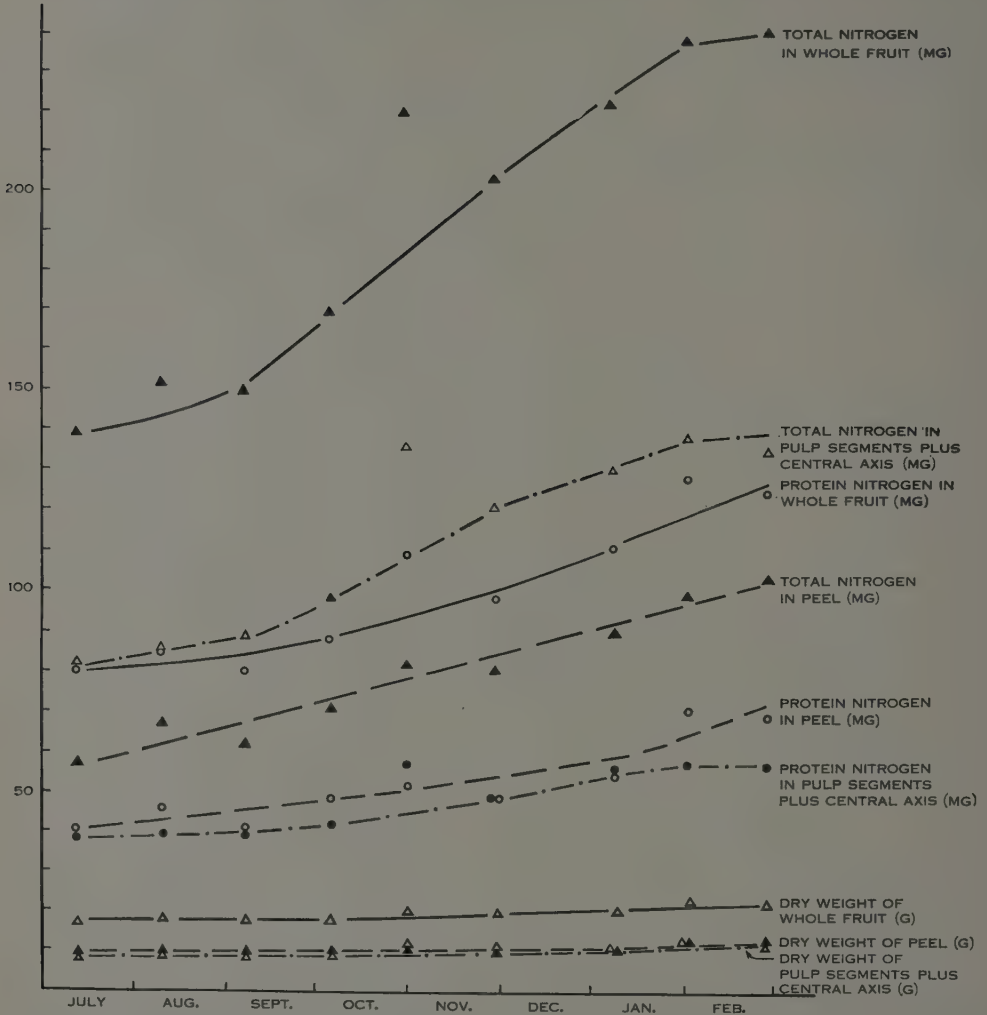


Fig. 12.—Changes in nitrogen content and dry weight during Stage III from mid July to maturity.

outer flavedo cells resembled the earliest stages in the development of lobed cells, and the inner flavedo cells showed transitional stages in the development of spongy tissue. Increase in pulp volume was due to the increased radial length of the septa, the tissue becoming spongier and thinner as the fruit matured. Juice sacs continued to enlarge during Stage III (Fig. 11) and the shorter-stalked sacs close to the

tangential wall of the segments were very compressed. The number of juice sacs per segment varied depending on the size of the segment and on the number of seeds developed in it. The number of sacs developed in three mature fruits of varying size is shown in Table 4.

Fresh weight and dry weight increased as long as the fruit was on the tree. Fresh weight increased at a similar rate in peel and pulp tissue (Fig. 11). The peel contributed slightly more to the dry weight of the maturing and mature fruit than the pulp (Fig. 12). Respiration rate per fresh weight, per fruit (Figs. 3 and 4) and per protein nitrogen, continued to fall throughout Stage III (0.06 to 0.03 mg CO₂/hr). Percentage moisture content in the peel showed a slight increase throughout Stage III and a slight increase in the pulp at the end of the period (Fig. 6). The accumulation of water and of sugar in the pulp remained in step during Stage III, the Brix reading (9.4°) and the sugar content of the juice being constant. The percentage acidity in the juice fell throughout the period (Fig. 7). Regreening of the outer peel began at the stem end during early January.

Changes in nitrogen content of the fruit during Stage III are shown in Figure 12.

TABLE 4
NUMBER OF JUICE SACS IN EACH PULP SEGMENT IN THREE MATURE FRUITS

| Fruit Radius (mm) | Number of Sacs in Each Pulp Segment | | | | | | | | | |
|----------------------|-------------------------------------|------|------|-----|-----|-----|------|-----|-----|-----|
| | | | | | | | | | | |
| 63.5 | 564 | 639 | 575 | 614 | 570 | 549 | 473 | 651 | 564 | — |
| 69.5 | 337 | 354* | 365* | 410 | 353 | 297 | 345 | 333 | 340 | 422 |
| 73.0 | 395 | 412 | 506 | 498 | 353 | 350 | 280* | 430 | 435 | 422 |

*One seed.

Total nitrogen increased for as long as the fruit was left on the tree, a similar situation to that found by Cameron, Appleman, and Bialoglowski (1935). This increase in nitrogen in fruit left on the tree may have been associated with synthetic processes leading to regreening. Increase in total nitrogen was slow in the early part of Stage III corresponding with the decreased growth rate. The pulp contributed most of the increase in nitrogen in the fruit during Stage III and was responsible for the initial check in nitrogen accumulation. Total nitrogen in the juice was approximately 75 per cent. of the pulp nitrogen in the ripe fruit.

Nitrogen accumulated at a steady but slower rate in the peel throughout the period. Protein nitrogen increased throughout Stage III but fell from 57.5 per cent. to 50.2 per cent. of the total nitrogen in the fruit by September; thereafter, protein nitrogen was only slightly higher than the soluble nitrogen per fruit. Practically equal amounts of protein nitrogen were found in the peel and the pulp; the pulp contained approximately 70.0 per cent. of the soluble nitrogen throughout Stage III. Protein nitrogen increased faster than soluble nitrogen in the whole fruit and in the

peel at the end of Stage III (Fig. 9). Increase in protein nitrogen accompanied increase in pulp moisture at about the rate expected for vacuolating cells—a sharp contrast to the situation in Stage II (Fig. 10). Total and protein nitrogen, expressed as percentage of dry weight, continued to fall until early September in the whole fruit, the peel, and the pulp and then began to rise gradually until the end of Stage III.

IV. ACKNOWLEDGMENTS

The author wishes to express gratitude to the late Mr. G. Linton and to Mr. K. Brushfield for making fruit samples available and for their cooperation throughout this investigation, which was part of the research programme of the Division of Food Preservation and Transport, C.S.I.R.O. Thanks are also due to Dr. R. N. Robertson for his assistance both during the course of the work and in the preparation of the manuscript; Mr. J. Casey, Mr. J. Smydzuk, and especially Miss M. Woodham for analytical and technical assistance; Mr. P. R. Maguire for the photography; Mr. G. G. Coote and Mr. A. G. Constantine of the Division of Mathematical Statistics, C.S.I.R.O., for statistical treatment; and Dr. J. R. Vickery, Dr. H. S. McKee, and Mr. E. G. Hall for their helpful criticism of the manuscript.

V. REFERENCES

- BAIN, J. M., and ROBERTSON, R. N. (1951).—The physiology of growth in apple fruits. I. Cell size, cell number, and fruit development. *Aust. J. Sci. Res.* **B 4**: 75–91.
- BARTHOLOMEW, E. T., and SINCLAIR, W. B. (1941).—Unequal distribution of soluble solids in the pulp of citrus fruits. *Plant Physiol.* **16**: 293–312.
- BEER, W. J., and PATTERSON, J. B. (1939).—The determination of nitrogen in cider and apple juice. *J. Soc. Chem. Ind., Lond.* **58**: 176–8.
- CAMERON, S. H., and APPLEMAN, D. (1934).—Total nitrogen in developing flowers and young fruits of the Valencia orange. *Proc. Amer. Soc. Hort. Sci.* **32**: 204–7.
- CAMERON, S. H., APPLEMAN, D., and BIALOGLOWSKI, J. (1935).—Seasonal changes in the nitrogen content of citrus fruits. *Proc. Amer. Soc. Hort. Sci.* **33**: 87–9.
- DUFRENOY, J. (1929).—A cytological study of water soluble and fat soluble constituents of citrus. *J. Agric. Res.* **38**: 411–29.
- FORD, E. S. (1942).—Anatomy and histology of the Eureka lemon. *Bot. Gaz.* **104**: 288–305.
- HAAS, A. R. C., and KLOTZ, L. J. (1935).—Physiological gradients in citrus fruits. *Hilgardia* **9**: 181–217.
- HALL, E. G. (1955).—Juice of oranges. *Food Pres. Quart.* **15**: 12–4.
- HARDING, P. L., and FISHER, D. F. (1945).—Seasonal changes in Florida grapefruit. Tech. Bull. U.S. Dep. Agric. No. 886.
- HARDING, P. L., and SUNDAY, M. B. (1949).—Seasonal changes in Florida tangerines. Tech. Bull. U.S. Dep. Agric. No. 988.
- HARDING, P. L., and SUNDAY, M. B. (1953).—Seasonal changes in Florida Temple oranges. Tech. Bull. U. S. Dep. Agric. No. 1072.
- HARDING, P. L., WINSTON, J. R., and FISHER, D. F. (1940).—Seasonal changes in Florida oranges. Tech. Bull. U.S. Dep. Agric. No. 753.
- HARVEY, E. M., and RYGG, G. L. (1936a).—Physiological changes in the rind of Californian oranges during growth and storage. *J. Agric. Res.* **52**: 723–46.
- HARVEY, E. M., and RYGG, G. L. (1936b).—Field and storage studies on changes in the composition of the rind of Marsh grapefruit in California. *J. Agric. Res.* **52**: 747–87.
- JOHANSEN, D. A. (1940).—“Plant Microtechnique.” (McGraw-Hill Book Co. : New York and London.)

- McKENZIE, H. A., and WALLACE, H. S. (1954).—The Kjeldahl determination of nitrogen. A critical study of digestion conditions—temperature, catalyst, and oxidizing agent. *Aust. J. Chem.* 7: 55-70.
- MARLOTH, R. H. (1950).—Citrus growth studies. II. Fruit growth and fruit internal quality changes. *J. Hort. Sci.* 25: 235-48.
- MARTIN, D., and LEWIS, T. L. (1952).—The physiology of growth in apple fruits. III. Cell characteristics and respiration activity of light and heavy crop fruits. *Aust. J. Sci. Res. B* 5: 315-27.
- REED, H. S. (1930).—The swelling of citrus fruits. *Amer. J. Bot.* 17: 971-82.
- ROBERTSON, R. N., and TURNER, J. F. (1951).—The physiology of growth in apple fruits. II. Respiratory and other metabolic activities as functions of cell number and cell size in fruit development. *Aust. J. Sci. Res. B* 4: 92-107.
- RYGG, G. L., and GETTY, M. R. (1955).—Seasonal changes in Arizona and Californian grapefruit. Tech. Bull. U.S. Dep. Agric. No. 1130.
- SAMISCH, Z., and COHEN, A. (1949).—Composition of oranges in Israel. Bull. Agric. Exp. Sta. Rehovot No. 51.
- SAUER, M. R. (1953).—Fruit sizes: need for adequate soil moisture in Feb.-May shown by Valencia measurements at Merbein. *Citrus News* 28: 138-9, 141.
- SCOTT, F. M., and BAKER, K. C. (1947).—Anatomy of Washington navel orange rind in relation to water spot. *Bot. Gaz.* 108: 459-76.
- SCOTT, F. M., SCHROEDER, M. R., and TURRELL, F. M. (1948).—Development, cell shape, suberization of internal surface, and abscission in the leaf of the Valencia orange, *Citrus sinensis*. *Bot. Gaz.* 109: 381-411.
- SINCLAIR, W. B., and RAMSAY, R. C., (1944).—Changes in the organic acid content of Valencia oranges during development. *Bot. Gaz.* 106: 140-8.
- TURNER, J. F. (1949).—The metabolism of the apple during storage. *Aust. J. Sci. Res. B* 2: 138-53.
- WAYNICK, D. D. (1927).—Growth rates of Valencia oranges. *Calif. Citrogr.* 12: 150, 164.
- WAYNICK, D. D. (1928).—Factors concerned in the growth of Valencia orange. *Calif. Citrogr.* 13: 200.
- WEBBER, H. J., and BATCHELOR, L. D. (1948).—"The Citrus Industry." Vol. I. (University of California Press: Berkeley and Los Angeles.)

EXPLANATION OF PLATES 1-4

PLATE 1

Fig. 1.—Transverse equatorial section of the ovary of the Valencia orange blossom (18. x. 54).

Hand section, stained with ruthenium red. $\times 85$.

- | | | |
|---|---|--|
| a, epidermis; | } | ovary wall or future peel of fruit |
| b, hypodermis; | | |
| c, oil glands forming in future flavedo zone; | | |
| d, future albedo tissue; | | |
| e, ring of vascular bundles in inner peel developing a branched network in ovary wall | | |
| f, loculus wall; | } | future pulp segment |
| g, juice sac primordia; | | |
| h, ovule; | | |
| i, septum between loculi | } | central axis |
| j, ring of vascular bundles; | | |
| k, outer axis tissue; | | |
| l, inner axis tissue | | |

Fig. 2.—Morphological changes occurring in the fruit during Stage I and in the early part of Stage II in fruit development. $\times 0.87$.

| | | | | |
|-------------|-----|------|-----|------------|
| a, blossom, | 18. | x. | 54; | } Stage I |
| b, | 1. | xi. | 54; | |
| c, | 29. | xi. | 54; | |
| d, | 13. | xii. | 54 | |
| e, | 20. | xii. | 54; | } Stage II |
| f, | 17. | i. | 55; | |
| g, | 14. | ii. | 55 | |

PLATE 2

Fig. 1.—Transverse section of blossom ovary (18. x. 54) showing cell division in (a) epidermis, (b) hypodermis, (c) developing oil gland, and (d) ovary wall cells. Hand section, stained with ruthenium red. $\times 350$.

Fig. 2.—Transverse section of peel flavedo zone showing continued formation and enlargement of oil glands during Stage I (29. xi. 54). Plugged stomata appear in epidermis. Hand section, stained with gentian violet. $\times 85$.

Fig. 3.—Transverse section of peel flavedo zone at the end of Stage I (20. xii. 54) showing further enlargement of oil glands. The central gland cells (Fig. 2) have been replaced by drops of oil. Hand section, stained with gentian violet. $\times 85$.

PLATE 3

Fig. 1.—Transverse section of central peel tissue (1. xi. 54) showing pectin thickening associated with the cell walls and intercellular spaces. Hand section, stained with ruthenium red. $\times 340$.

Fig. 2.—Transverse section of central peel tissue (29. xi. 54) showing increasing amounts of pectin associated with each cell. The outer limit of the pectin layer around each cell is regular in outline and the inner limit slightly irregular. Hand section, stained with ruthenium red. $\times 340$.

Fig. 3.—Transverse section of central peel tissue at the end of Stage I (20. xii. 54) showing further increase in pectin associated with each enlarging cell. The inner limit of the pectin layer appears very irregular. Air spaces are small. Hand section, stained with ruthenium red. $\times 340$.

Fig. 4.—Transverse section of (a) inner wall tissue and (b) loculus of blossom ovary (18. x. 54), showing developing juice sac primordia, (c). Hand section, stained with ruthenium red. $\times 340$.

Fig. 5.—Transverse section of pulp segment showing development of club-shaped juice sacs (29. i. 54). Hand section, stained with gentian violet. $\times 340$.

Fig. 6.—Transverse section of pulp segment showing development of stalked juice sacs by the end of Stage I (20. xii. 54). Hand section, stained with gentian violet. $\times 85$.

PLATE 4

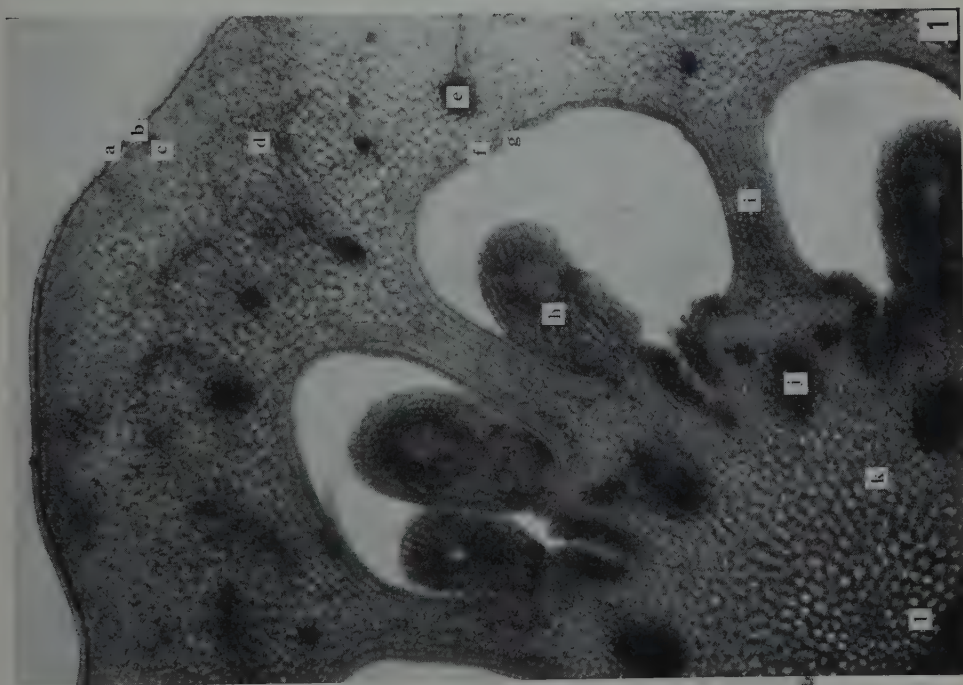
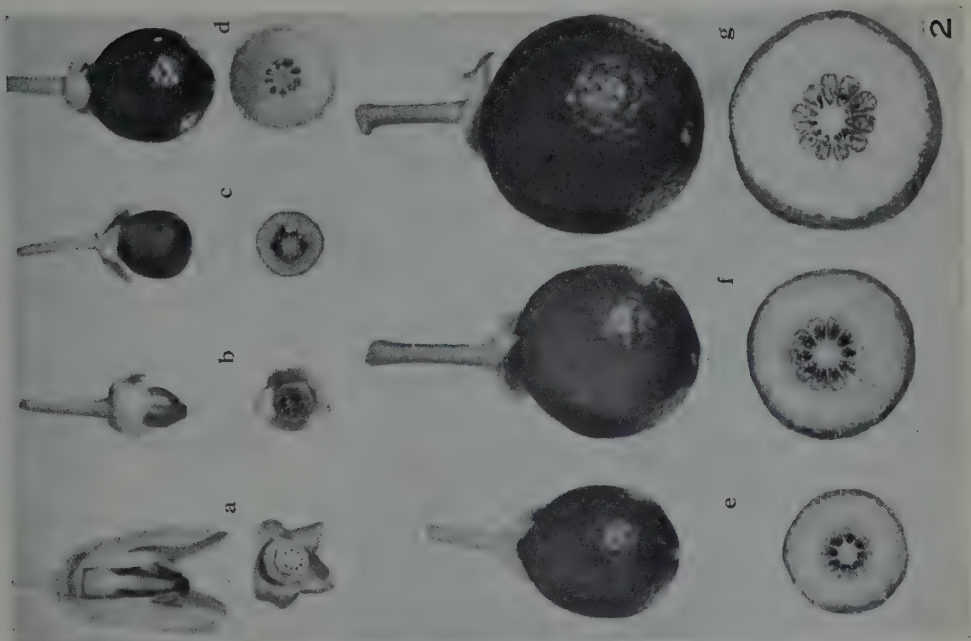
Fig. 1.—Macerated central peel tissue showing cells developing small protuberances (17. i. 55). Stained with gentian violet. $\times 400$.

Fig. 2.—Macerated central peel tissue showing increased cell size, lengthening of cell protuberances and increase in pectin (14. ii. 55). Stained with gentian violet. $\times 400$.

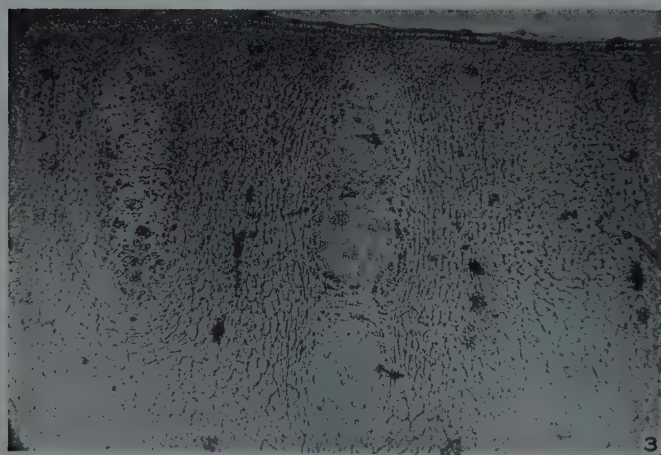
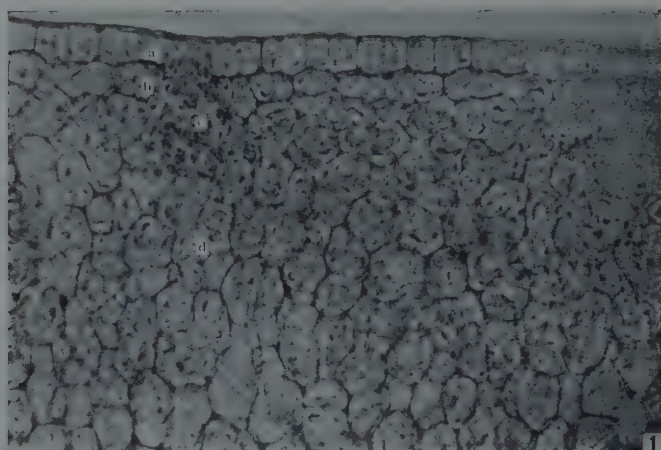
Fig. 3.—A typical "spider-like" cell taken from macerated spongy central peel tissue at the end of Stage II (11. xii. 55). Stained with gentian violet. $\times 400$.

Fig. 4.—Partly macerated central peel tissue of mature fruit showing "spider-like" cells forming a spongy network. Cells are much distorted and lined with pectin (10. i. 56). Stained with gentian violet. $\times 400$.

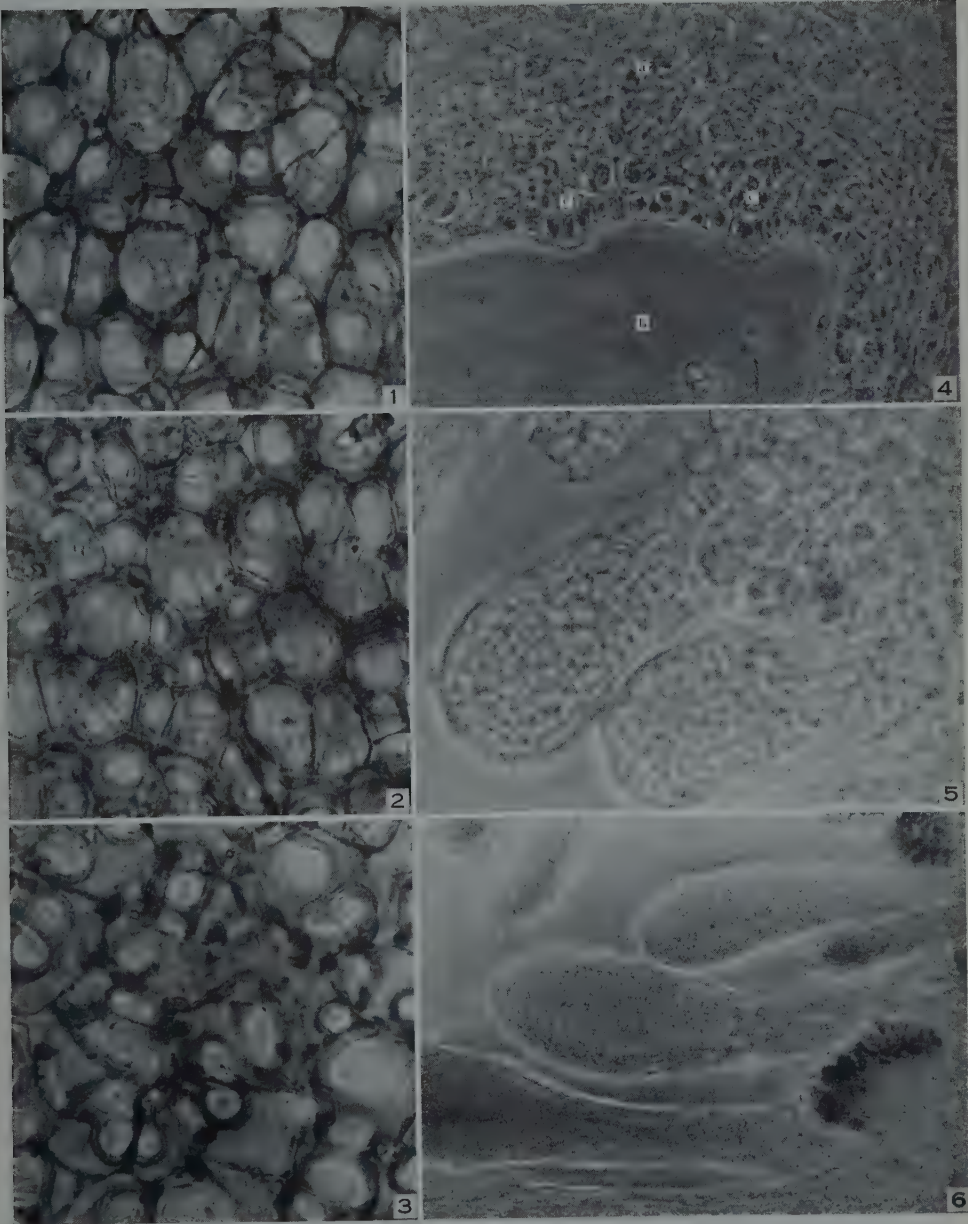
CHANGES IN THE DEVELOPING FRUIT OF THE VALENCIA ORANGE



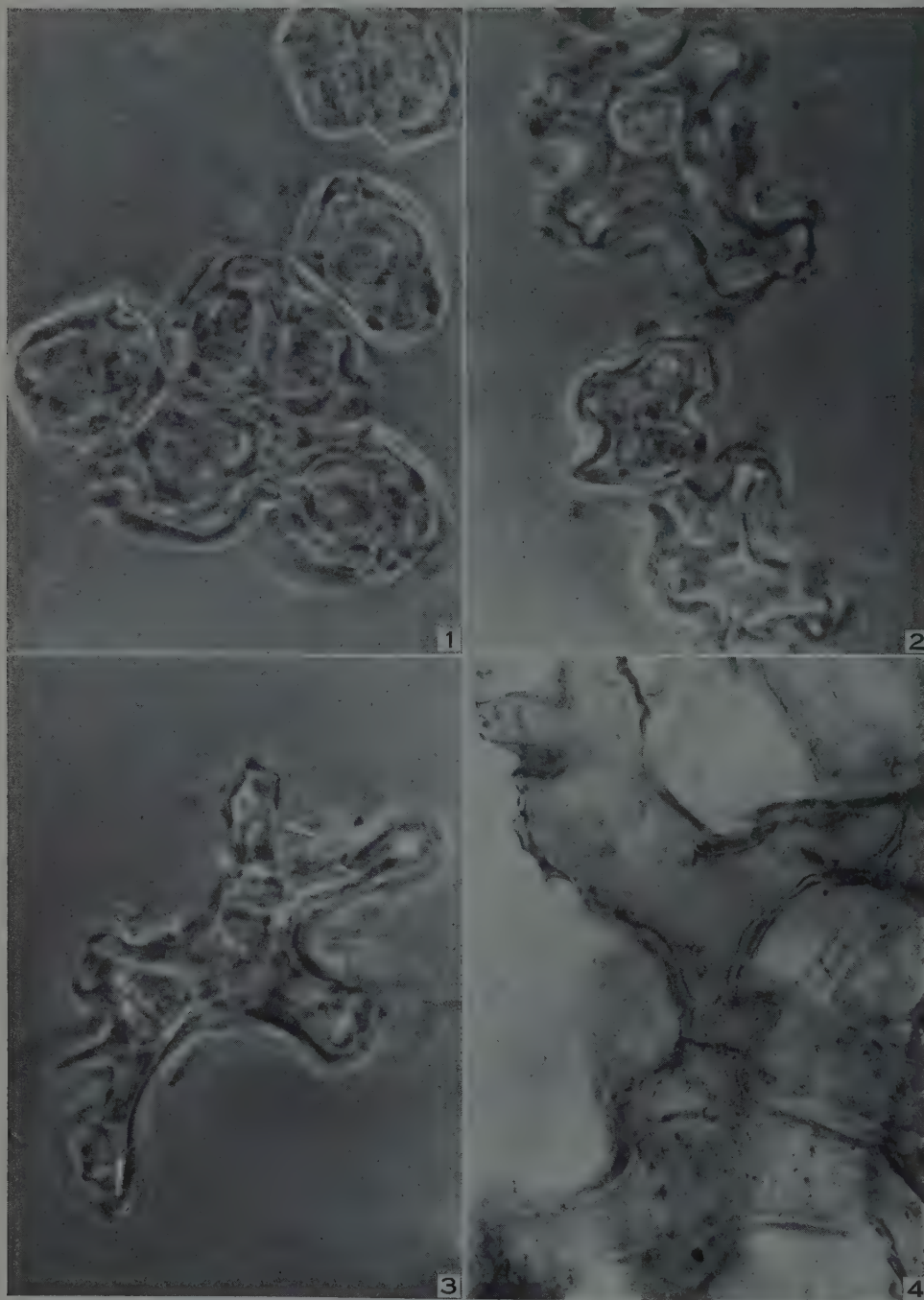
CHANGES IN THE DEVELOPING FRUIT OF THE VALENCIA ORANGE



CHANGES IN THE DEVELOPING FRUIT OF THE VALENCIA ORANGE



CHANGES IN THE DEVELOPING FRUIT OF THE VALENCIA ORANGE



THISMIA RODWAYI F. MUELL. AND ITS ENDOPHYTE

By ETHEL I. McLENNAN*

[Manuscript received September 3, 1957]

Summary

The morphology of *Thismia rodwayi*—a holosaprophytic plant belonging to the Burmanniaceae—is described, together with an account of its associated mycorrhiza. Evidence is presented of the liberation of an osmic-staining fat from the fungal hyphae into the cells of the higher plant. Subsequently these globules disappear and are replaced by a complex polysaccharide resembling glycogen.

The appearance of osmic acid-staining globules in the lumen of the cells and their ultimate disappearance is shown to be a phenomenon common to the complete saprophyte and to green mycorrhizal plants. It is, therefore, unrealistic to suggest that this arbuscular type of mycorrhiza should be interpreted as an example of parasitism, as is so often the case in the relevant literature.

I. INTRODUCTION

In 1890 Rodway sent to the National Herbarium, Victoria, a specimen of a plant collected by him in Tasmania, which proved to be a new species of *Thismia* and which was named *T. rodwayi* by Mueller (1890, 1891). It was not until 1935 that plants of this species were found on the Australian mainland. In October of that year Miss D. Coleman discovered the plant flowering in Sherbrooke Gully in the Dandenong ranges (1000 ft) some 25 miles from Melbourne (Coleman 1935). Although the area was searched the following year, no sign of the plants could be found, but in October 1940, a further gathering was made in the same locality (Coleman 1941). Our knowledge of the geographical range on the Australian mainland of this rare plant was extended when flowering specimens were found on the Black Spur (close to Marysville) in the Healesville ranges 50 miles from Melbourne at an altitude of 1800 ft.

For Schlechter (1921) the genus *Thismia* Griff. is characterized by free perianth lobes in contrast to *Sarcosiphon* Bl. where the inner perianth lobes are connate to a mitre. The Australian plants show the tops of the inner lobes locked together or perhaps slightly connate and so Schlechter changed the name to *Sarcosiphon rodwayi* (F. Muell.) Schl.

Jonker (1938) in a monograph of the family Burmanniaceae—the greater number of its members are saprophytic in habit—suggests that the family should be divided into two tribes, Burmannieae and Thismieae, according to the flower structure. The latter tribe is characterized by a one-celled ovary with three placentas, and a short thick style. He further divides the Thismieae into the Oxygyneae with three stamens and the Eu-thismieae with six stamens to which the genus *Thismia* is assigned. He regards *Thismia* and *Sarcosiphon* as synonymous, for in the latter genus all gradations between free and united perianth lobes are found, so that the Australian plant is now cited under the name conferred upon it by Mueller in 1890—*Thismia rodwayi*.

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Cheeseman (1908) gave the name *Bagnisia hillii* to a plant found in New Zealand which is now considered identical with the Australian plant; so this name falls into synonymy.

The Thismieae inhabit the tropics of both hemispheres and are found in deep humus in forest areas. Two closely connected species, *T. rodwayi* and *T. americana* Pfeiffer, are the exceptions to this tropical distribution. *T. rodwayi* is found only in Tasmania, Victoria, and New Zealand, while *T. americana* is known only from an open prairie near Chicago. Jonker recognizes that the differences between these two species are very small and he suggests that perhaps they are identical. The American plant is known only from the original gathering as described by Pfeiffer (1914).

II. MORPHOLOGY OF THISMIA RODWAYI

The entire plant is subterranean in habit; the only part appearing above ground are the flowers and even these are half embedded in the rich humus or they may complete their cycle without reaching the surface. Coleman (1935) found "a fully expanded flower completely underground". The outer surface of the plants is invested with a cobwebby coating of fungal hyphae intermixed with the humus.

The subterranean parts of the plant consist of sparingly branched, cylindrical, colourless axes 1-1.5 mm in diameter; these ramify in the deep humus in which the plants grow. When removed and washed in running water they are seen to be glabrous and translucent white in appearance. At intervals short flower-bearing shoots arise in the axil of a small colourless scale leaf and, supporting one or two similar leaves, they terminate in a flower bud (Plate 1, Figs. 1 and 3).

There has been much discussion in the literature as to the nature of these subterranean axes in the saprophytic thismias (Groom 1895; Ernst and Bernard 1909; Pfeiffer 1914). Transverse sections of the more or less horizontally-running axes of *T. rodwayi* show a well-marked stelar region with a centrally placed xylem strand and exarch position of the protoxylems so characteristic of root structure (Plate 1, Fig. 2). The stele is bounded by an endodermis of large cells enclosing a pericyclic layer. According to the size of the root there are from four to eight thick-walled xylem elements in the vascular strand. All are approximately equal in diameter, but occasionally the protoxylem vessels appear smaller. The phloem tissue is not well differentiated; it is difficult to distinguish it from the parenchyma cells binding the vascular tissue together (Plate 1, Fig. 2).

In longitudinal view the xylem strand shows strong lignification in a scalariform pattern with the protoxylem carrying annular thickenings (Plate 4, Fig. 3). The narrow elongated cells with dense granulated contents appear to be sieve tubes; sieve plates seem to be lacking.

Pfeiffer (1914), describing the anatomy of *T. americana*, states that the central strand "is not lignified and that the spiral markings are very fine".

The stele is surrounded by a cortex of parenchymatous cells, seven or eight layers deep. The outer cortical layer referred to by Groom (1895) as the "exocortex" consists of larger cells somewhat elongated in the radial plane and without intercellular spaces; the layer of cortical cells immediately beneath them is composed of cells

smaller than those of the general cortex and appearing conspicuous in sections because of their contents. Groom called this layer the "limiting layer" (Plate 2, Fig. 3). The surface layer of the root resembles an epidermis rather than a piliferous layer. It consists of large, somewhat papillose cells with the outer protruding walls slightly thickened; no traces of root hairs have been seen. Humus particles lodge in the concavities on the outer surface of this layer and persist after thorough washing; they can be seen in prepared sections. The epidermis is free from fungal infection except at points of penetration by the mycorrhizal form, but all other cells of the cortex are occupied by the fungus. The endodermis and the stele are always free of hyphae.

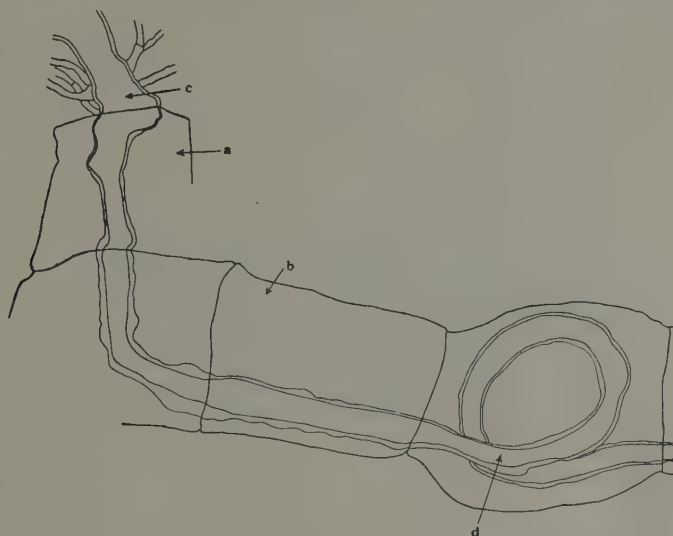


Fig. 1.—Coarse hypha entering root through an epidermal cell and passing directly into the exocortex where it runs tangentially and coils in the cells of this layer. $\times 200$. *a*, epidermal cell; *b*, exocortex; *c*, hyphae external to the root; *d*, beginning of coil.

The flowering axes in transverse section show the xylem tissue to consist of three or more bundles arranged in a cylinder (Plate 1, Fig. 4). Each bundle has vessels of the scalariform type and protoxylem elements. The phloem tissue is collateral in position, but no sieve plates have been observed either in transverse or in longitudinal section. The presumed sieve tubes are narrow with granular protoplasmic contents. The stele is surrounded by an endodermis of large cells with pads of thickenings on their radial walls.

The scale leaves have a single simple leaf trace which runs through the greater part of their length.

III. THE MYCORRHIZA

The sequence of development following infection has been observed in longitudinal sections taken along the length of the root extending to the root apex.

The hyphae of the fungus exterior to the roots are coarse, thick-walled, and septate. They gain entrance into the roots either by direct penetration of the epidermal cells or by forcing their way between them (Fig. 1). Once inside the root tissues, they are unseptated and pass directly through the epidermal layer into the layer of large cells beneath (exocortex). Here they run tangentially passing from cell to cell without any constriction as they go through the wall. A single penetration is responsible for an extensive "infection" of the root tissue (Plate 2, Fig. 1).

Branches arise in each cell from these travelling hyphae. They are coarse, often of the same diameter as the parent hypha, and form very conspicuous coils of large thick-walled hyphae from which a coarse and complex fungal network arises and almost fills the interior of these cells (Fig. 2 and Plate 2, Fig. 3).

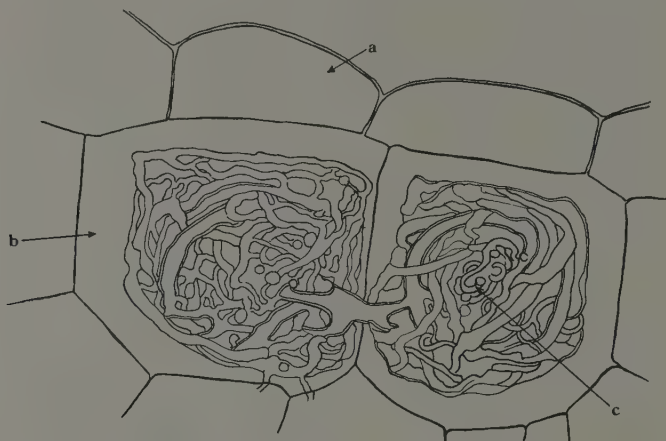


Fig. 2.—Coils of hyphae in the cells of the exocortex. In this layer the hyphae are coarse and thick-walled. $\times 275$. *a*, epidermis; *b*, exocortex; *c*, hyphal coils.

The cells of the layer underneath the exocortex—the limiting layer—are smaller; on the average three cells extend across the inner tangential wall of the larger exocortical cells. A hypha from the large cell penetrates one or more of the cells lying underneath it; these hyphae coil and branch again to form a network of much finer thin-walled and highly protoplasmic threads (Fig. 3). Lateral penetration of the radial walls carries the fungus into the neighbouring cells constituting this layer (Plate 2, Fig. 3).

The cells of the limiting layer in turn provide the hyphae which pass downwards into the rest of the cortex. The cortical tissue consists of large thin-walled closely packed cells without intercellular spaces. Some of the cortical cells lying close to the limiting layer may show large approximately globular vesicles, $50\text{--}70\ \mu$ in diameter, similar to those which have often been recorded for the arbuscular endophytic type of mycorrhiza (Plate 2, Figs. 2 and 4). The surrounding wall becomes thickened and the dense protoplasmic contents contain numerous nuclei. Some of the vesicles eventually appear to be filled with small rounded nucleated cytoplasmic bodies resembling spores (Plate 3, Fig. 1).

Thismia rodwayi was collected at flowering time and the cortical cells in preparations made from this material showed many communicating hyphae passing

through the walls of neighbouring cortical cells. The hyphal walls were thin and they showed no distortion at their points of penetration or passage through the cell walls. In the interior of the cells the hyphae form a network of branches. When



Fig. 3.—A cell of the exocortex and the underlying cells of the limiting layer. Hyphae from each exocortical cell penetrate into several of the smaller cells beneath it. The coils in this layer consist of thin-walled fine hyphae. $\times 300$. *a*, exocortical cell; *b*, cells of limiting layer; *c*, coils of fine hyphae.

the mycorrhizal association is in its early stages in appropriately fixed material, e.g. with Fleming's fixative, the hyphae can be seen to be packed with a fatty substance which stains intensely black with osmic acid. Sometimes these fatty deposits are so concentrated that the outline of the hyphal threads becomes distorted,

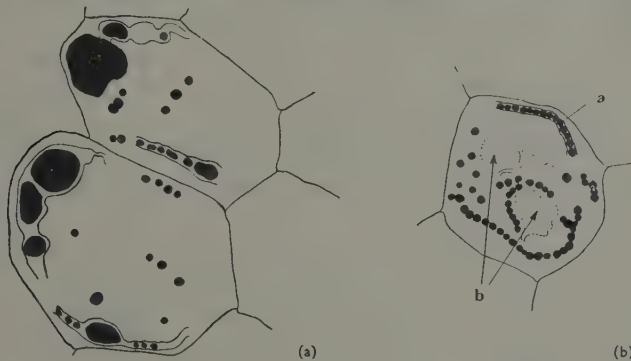


Fig. 4.—Cortical cells of *Thismia rodwayi*. $\times 215$. (*a*) Oil globules distorting outline of hypha. (*b*) *a*, oil globules in hypha and lying free in the cell, *b*, position of digested ends of hyphae.

but more often they occur in a regular series of globules along the length of the hypha without any disturbance of its shape (Fig. 4 and Plate 3, Fig. 3). At this stage the lumen of the cell is free of material reacting with osmic acid, other than that contained in the hyphal system itself.

In some cells, in which "infection" is presumably at a later stage, changes occur in the hyphae present. At certain points the ends of hyphal branches swell up and it is at these points that the so-called digestion starts. The inflation of the hyphal tips occurs usually at three or more points in the cell; the surface of these swellings becomes mammillated (Fig. 5), each mammilla forming a type of sporangiole comparable with the sporangioles characteristic of the typical arbuscular-sporangiole type of mycorrhiza.

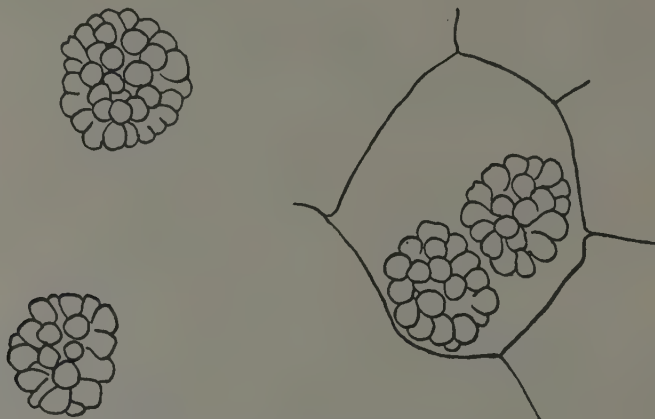


Fig. 5.—Cortical cell of *Thismia rodwayi* with the mammillated ends of hyphae before digestion. $\times 350$.

Janse (1897) records for another saprophytic species of the Burmanniaceae (*Thismia clandestina* Miq.) the presence of sporangioles in the inner cortical layers, and figures them. Groom (1895), discussing *T. aseroe* Beccari, states that the hyphae swell out to form "a bladder which gradually assumes a spherical form". At this stage "it is filled with a densely staining mass of cytoplasm. . . . The bladder is now mature, and the further changes in it are associated with its waning vitality".

Van der Pijl (1934), discussing the endophyte of *Burmannia candida* Engl., a saprophytic species, likens the type of digestion to that characteristic of the Orchidaceae, to which the term "tolypophage" has been given. He states that the hyphal knots are not so thick as those found in the orchid cells, and his description is generally unconvincing.

In *Thismia rodwayi* the wall of the bladder becomes diffuse and weakened and finally nothing remains but a shrunken mass; this change is accompanied by the appearance of a large number of the black-stained globules, formerly restricted to the hyphal system, in the lumen of the *Thismia* cells (Plate 3, Fig. 2). Their uniform size and the large numbers present give a very characteristic appearance to the cortical tissue at this stage.

In fresh material the collapsed and shrunken hyphal bladders appear deep yellowish brown in colour and of a rosette form, but in fixed and sectioned material they react to haematoxylin stains (Plate 3, Figs. 2 and 4).

After the discharge of the fat globules into the cells a substance of a polysaccharide nature begins to accumulate, at first in small amounts (Plate 4, Fig. 2),

but later in quantity. It can be readily observed in sections stained with iron-alum haematoxylin and safranin. The fat globules remain black, the hyphal complex stains the familiar purplish colour of the haematoxylin, while this last substance to appear stains with the safranin. The accumulation of this secondary substance continues at the expense of the fat globules which first of all undergo a reduction in size and finally gradually disappear from the cell lumen. An examination of the amorphous mass of polysaccharide material collecting in the cells at the later stages of digestion shows evidence of the degradation of the fat globules in the process of the formation of the accumulating polysaccharide (Plate 4, Figs. 2 and 4). This change occurs throughout the cortical region but the density of the precipitation of the polysaccharide as seen in fixed and sectioned material reaches its peak in the two or three cortical layers abutting on to the stele, so at this stage a section of the root taken in the median plane shows these cells forming a very characteristic sheath around the stele (Plate 4, Fig. 1).

If the sections are stained with a 1 : 1000 aqueous solution of the fluorescent stain acridine orange for 2 min, the distribution of the polysaccharide in the sections is beautifully outlined. The concentration effect of the stain results in a polychromatic effect.

Numerous mucilage cells with raphides occur in any position among the cortical cells. The stele itself is free of hyphae.

In sections of older roots taken at a late stage in the development of the plant the appearance of the tissues changes. The coarse coiled hyphae of the exocortex retain their form, but the finer branches appear shrunken. In both types of hyphae their contents have disappeared, the walls of the hyphae stain densely with haematoxylin and other dyes. The cortical cells now contain only the shrunken remnants of the hyphal component. Typically there are two or three rosette-like bodies, representing points where the digestion was active, and the shrivelled remains of the hyphae. There is a complete absence of osmic-blackened fat globules and there is no trace of the polysaccharide substance which is so conspicuous in younger and active roots (Plate 3, Fig. 4).

IV. IDENTIFICATION AND DISTRIBUTION OF THE POLYSACCHARIDE

Sections of roots treated with Lugol's iodine solution show this substance to take on a reddish brown colouration. Swanson (1948) has shown that the iodine colour given by polysaccharides is mainly dependent on the length of the chain structure. Chains from 4 to 6 glucose units long give no colour reaction with iodine, those from 8 to 12 units give a red colour, followed by transitional colours until a length of from 30 to 35 units is reached when the iodine colour becomes blue. The relationship between chain length and iodine colour has been applied to the branched polysaccharides. It is estimated that amylopectin has some inner branches as long as 10 units, while glycogen contains some inner branches as long as 8 units. The colour reaction with iodine of the *Thismia* material suggested that the polysaccharide in the roots might possibly be one of the glycogens.

Histochemical tests for the detection of glycogen in tissues were next resorted to (Bensley 1939; Carpenter, Polonsky, and Menten 1951; Pearse 1954). From

material fixed in alcoholic Fleming's fixative both transverse and longitudinal root sections were stained according to Best's carmine method. The stain was prepared as follows: Grubler's carmium rubrum optimum (2 g), potassium carbonate (1 g), and potassium chloride (5 g) were dissolved in distilled water (60 ml). The solution was boiled gently, allowed to cool, and then 20 ml of concentrated ammonia were added. For use, to 2 parts of this stock solution were added 3 parts of concentrated ammonia and 3 parts of pure methanol.

After staining the slides with Heidenhain's iron-alum haematoxylin technique they were stained with the diluted Best's carmine solution for 15–20 min, then differentiated in a solution of ethanol (80 parts), methanol (40 parts), and distilled water (100 parts), cleared, and mounted in balsam.

A duplicate series of slides was prepared. One series, which served as a control, was subjected to treatment by saliva for $2\frac{1}{2}$ hr while incubated at 32°C before staining. After this treatment the saliva was washed away and the slides were then stained by the haematoxylin and carmine method. The other series was similarly stained but without pretreatment by saliva. As a further control, slides of rat's liver were prepared and stained by the same method. The liver samples were obtained from rats which had received, in addition to their normal diet, 20 g of glucose each daily for 6 days.

The "undigested" slides showed that the substance under examination stained with the carmine stain a red colour. This colour was particularly conspicuous in the cylinder of cortical cells (about two cells wide) directly against the stele, and the material occupied the greater part of the cell lumen (Plate 4, Fig. 1).

Passing outwards through the cortex, this substance, staining in the same way, occurred in many of the cortical cells. Here, however, the cells still contained many fat globules and some undigested hyphae. The amount of the polysaccharide present varied according to the degree of digestion of the hyphal complex. On the whole it increased in quantity passing inwards through the cortex towards the stele. However, it was noticed that the cells close to the limiting layer often showed a fairly heavy accumulation of it.

The "predigested" slides provided a striking contrast. The two-layered cylinder of cortical cells adjacent to the stele showed no trace of the polysaccharide nor could any be detected throughout the cortical region (Plate 4, Fig. 3, cf. Fig. 1).

The sections of rat's liver gave a confirmatory result in so far as the glycogen granules stained red with the carmine.

Root sections were also stained with the periodic acid-Schiff method. For details of solutions used see Pearse (1954). Slides so stained gave a purplish red reaction in the cells where the polysaccharide was precipitated. The reaction of the polysaccharide to various staining methods was as follows:

| <i>Method</i> | <i>Result</i> |
|----------------------------|----------------------|
| Lugol's iodine | Reddish brown colour |
| Saliva treatment | Labile |
| Best's carmine, undigested | Red colour |
| Best's carmine, digested | — |
| Periodic acid-Schiff | Purplish colour. |

The evidence points to the fact that the substance under discussion might in fact be glycogen. It should be stressed that staining methods will not in themselves resolve with certainty the character of the cell substance. All one can say is that we are here dealing with a complex polysaccharide. Microchemical tests must be made to establish the nature of the material with complete certainty; lack of material has prevented this from being done.

The only certain record of the occurrence of glycogen in Phanerogams so far as the author is aware is that noted by Morris and Morris (1939). Hassid and McCready (1941) obtained a polysaccharide from the seeds of maize which had the properties commonly associated with glycogen. They attempted to identify this substance by comparing its rate of enzymatic hydrolysis and its cupric chloride crystallization patterns with those of glycogens obtained from animal sources, and they concluded that the polysaccharide isolated from the corn was apparently identical with glycogen.

V. DISCUSSION

Thismia rodwayi is entirely devoid of chlorophyll and, like all other holosaprophytes, it contains an endotrophic mycorrhiza. The present paper proposes to show that in all probability this association is not a case of parasitism of the fungus on a host plant, but is a true symbiosis. The organic materials in host and fungus can only be obtained from the soil. The absence of root hairs and the presence of hyphal filaments entering the plant from the soil suggest that these materials are obtained from the soil by the fungus. This is in line with the first appearance of storage materials (fat) in the cells of the fungus rather than in the cells of the other partner. In the "digestion" of the hyphal ends nitrogenous material is no doubt also liberated into the cells of *Thismia*, but this cannot be easily demonstrated.

McLennan (1926, see Plate 2, Figs. 6, 7, 8, and 9; and Plate 3, Figs. 16 and 23 of that paper) was the first to point out the exchange of osmic-staining fat globules from a fungus to a higher plant through a mycorrhizal complex and to discuss the significance of this exchange to the metabolism of the green plant. Fraser (1931) observed a similar phenomenon in two species of *Lobelia*. The mycorrhiza occurring in association with these plants is of a very special type, entirely intercellular in position in the roots. It is nevertheless truly endophytic and it differs from the typical ectotrophic arrangement. It is considered by Fraser to be an obligate association. In the mid cortex of "the roots the fungal hyphae are much extended by the presence of reserve food which has accumulated in much larger droplets than elsewhere" (Fraser 1931, see Figs. 15, 17, 18). This stage of the mycorrhiza is succeeded by what Fraser has termed "the period of fungus depletion". This is characterized by the appearance in the cytoplasm of the cortical cells of numerous small droplets of a reserve food similar in staining properties to those of the fungal hyphae. Between these cells, the hyphae appear compressed and have lost practically all their reserve food. The depletion is at first most marked in the innermost part of the fungal zone and the hyphae between the cells of the inner cortex are so crushed as to be practically invisible. This process of depletion goes on progressively towards the outer cortex until finally all reserve food is seen to have disappeared from the

fungus. The droplets of reserve food in the fungal hyphae during the fungal enlargement period and the droplets which appear in the cortical cells of the root during the subsequent period of fungal depletion stained a dense black with osmic acid. Fraser states that "this reserve food is entirely soluble in chloroform and stains in Sudan III, and in sections of fresh material collects in droplets around the section".

McLuckie and Burges (1932) described the mycorrhizae associated with *Eriostemon crowei* F. Muell. and some other genera of the Rutaceae. They were characterized by the presence of arbuscules, sporangioles, and vesicles of the type of mycorrhiza which is so widespread, and so common in many herbaceous angiosperms. The sporangioles in their early stages are completely filled with cytoplasm and enclosed fat globules which again stain black with osmic acid. The sporangioles ultimately undergo "digestion" and the authors go on to say "... their contents, cytoplasm and fat, lie in the cell-cavity. The fat globules lie in the mass of fungal cytoplasm derived from the sporangioles and the hyphal branches forming the original arbuscule collapse and appear colourless and practically empty. . . . At this stage the cell-contents are a dense mass of finely granular fungal cytoplasm, numerous fat globules, fragments of shrivelled hyphae" (McLuckie and Burges 1932, see Figs. 8, 10, 12, 13, and 17 of that paper).

At a later stage in this mycorrhizal association the authors state that "the large globules of fatty material which accumulate in the cells of the host root, owing to liberation from the ruptured sporangioles, are ultimately removed, presumably by the host".

In all of the cases cited above, even when the endotrophic mycorrhiza is not always of the same type, there has been complete agreement that the osmic acid-staining globules, first present in the hyphae, are later set free and accumulate in the plant cell and finally disappear as a further stage of metabolism is reached. Notwithstanding this basic agreement, there has been much discussion in the literature concerning the interpretation of the relationship between the green plant and the fungal associate when it is in the form of an endotrophic mycorrhiza (excluding Orchidaceae).

Burges (1936) in a general discussion on mycorrhiza concludes with this statement: "The presence of the fungus in a mycorrhizal association is to be regarded as an example of controlled parasitic attack, and has no mutualistic significance. The fungi concerned are weak pathogens whose activity is curbed by the reactions of the host cells".

In this way he dismisses an earlier statement (McLuckie and Burges 1932): "While the entrance of the fungus to the host root indicates parasitism, the fact remains that the endophyte is controlled and largely digested by the host cells which receive considerable quantities of fat and, therefore, in the final resort, the higher plant is parasitic upon its endophyte".

Again Burges (1939), although now discussing the mycorrhizae of orchids, points out that Bernard (1904) has suggested that the orchids were "plantes atteintes d'une maladie parasitaire chronique qui commence à la germination et persiste en général jusqu'à l'état adulte", and that it was his opinion that Bernard's remarks might be extended to all endotrophic forms.

Harley (1950) tabulates a number of examples of endotrophic mycorrhizae and among other characters he notes the type of digestion characteristic of them. He remarks: "Digestion or disintegration of the hyphae occurs within the living host tissues at some stage of development. Such breakdown of the fungal hyphae demonstrates a tendency on the part of the host to eliminate the endophyte, such as occurs in some cases of parasitic attack. . . . Digestion has also been frequently assumed to indicate an intake of material by the host from the fungus and hence by the living system from the soil. Such an assumption is quite unjustified".

Harley goes on to say that the occurrence of digestion does indicate the gain by the host of material from the fungus but that it does not demonstrate a gain by the living system due to the action of a fungus at the expense of the environment. Such a gain, he says, can only occur if at least one of a set of conditions is fulfilled. Among these conditions he cites "the conduction of material into the host by the fungus from the soil. This requires hyphal connections with the soil in significant quantity and an external mycelium absorbing soil substances". In his discussion of the vesicular-arbuscular type of mycorrhiza, Harley admits that the available evidence suggests that there are adequate hyphal connections with the soil for the fungus to fulfil a role of intermediary in absorption. Applicable to this discussion, although in this case the mycorrhiza was of the ectotrophic type, are the results from some recent experiments conducted by Melin and Nilsson (1950) on the transfer of radioactive phosphorus to pine seedlings. They showed that "the radioactive component had been transported from its source to the mycorrhiza by the *Boletus* hyphae . . . and that the isotope had also been transferred to the cells of the higher symbiont from the fungal partner".

Finally, Harley refers to the fact that some of the Burmanniaceae seem to possess the vesicular-arbuscular type of mycorrhiza, and that it is present in the saprophytic Pteridophyte prothalli. This leads one "to suppose such infection is not likely always to be parasitism".

The arguments used in interpreting the vesicular-arbuscular type of endotrophic mycorrhiza as essentially parasitic in character can no longer be justified for the following reasons.

Firstly, adequate hyphal connections between the soil and the higher plant have been shown to exist for many mycorrhizae and this is true also for *Thismia rodwayi*. Secondly, the appearance of oil globules in the fungal hyphae during the early establishment of the root mycorrhizae, followed by their appearance in the lumen of the root cells at digestion and their ultimate disappearance, has now been shown to be a phenomenon common to the complete saprophyte and to green mycorrhizal plants. If this exchange of material is accepted as passing from the fungus to the flowering plant in the saprophyte it becomes illogical to attempt to explain an exactly similar phenomenon in the roots of the green plants in any but the same way and quite unrealistic to suggest for the latter group of plants an explanation involving the idea of fungal parasitism. Whatever interpretation is accepted for one type of plant must be applicable to the other, and it is difficult, in fact impossible, to substantiate any argument involving this idea of parasitism for the completely saprophytic group as exemplified by *T. rodwayi*.

VI. REFERENCES

- BENSLEY, CAROLINE M. (1939).—Comparison of methods for demonstrating glycogen microscopically. *Stain. Tech.* **14**: 47–52.
- BERNARD, N. (1940).—Recherches experimentales sur les orchidées. *Rev. Gén. Bot.* **16**: 405.
- BURGES, A. (1936).—On the significance of mycorrhiza. *New Phytol.* **35**: 117–219.
- BURGES, A. (1939).—The defensive mechanism in orchid mycorrhiza. *New Phytol.* **38**: 273–83.
- CARPENTER, ANNA M., POLONSKY, BEATRICE, and MENTEN, MAUD L. (1951).—Histochemical distribution of glycogen. i. Evaluation of methods. *Arch. Path.* **51**: 480–5.
- CHEESEMAN, T. F. (1908).—*Bagnisia hillii* Cheeseman. *Kew Bull.* **1908**: 419–21.
- COLEMAN, DOROTHY G. (1935).—*Sarcosiphon rodwayi* in Australia. *Vict. Nat.* **52**: 163–6.
- COLEMAN, DOROTHY G. (1941).—Further notes on “Fairy Lanterns”. *Vict. Nat.* **57**: 167–8.
- ERNST, A., and BERNARD, L. (1909).—Äussere und innere Morphologie von *Thismia javanica* J. J. Smith. *Ann. Jard. Bot. Buitenz.* **8**: 36–47.
- FRASER, LILIAN (1931).—An investigation of *Lobelia gibbosa* and *Lobelia dentata*. I. Mycorrhiza, latex system and general biology. *Proc. Linn. Soc. N.S.W.* **56**: 497–525.
- GROO, P. (1895).—On *Thismia aseroe* Beccari and its mycorrhiza. *Ann. Bot.* **9**: 327–61.
- HARLEY, J. L. (1950).—Recent progress in the study of endotrophic mycorrhiza. *New Phytol.* **49**: 213–47.
- HASSID, W. Z., and MCCREADY, R. M. (1941).—The molecular constitution of glycogen and starch from the seed of sweet corn (*Zea mays*). *J. Amer. Chem. Soc.* **63**: 1632–5.
- JANSE, J. M. (1897).—Les endophytes radicaux de quelques plantes javanaises. *Ann. Jard. Bot. Buitenz.* **14**: 53–201.
- JONKER, F. P. (1938).—A monograph of the Burmanniaceae. Med. Bot. Mus. en Herb. van de Rijksuniversiteit te Utrecht, No. 51.
- MCLENNAN, ETHEL I. (1926).—The endophytic fungus of *Lolium*. ii. The mycorrhiza in the roots of *Lolium temulentum* L. with a discussion on the physiological relationships of the organism concerned. *Ann. Bot.* **40**: 43–68.
- McLUCKIE, J., and BURGES, A. (1932).—Mycotrophism in the Rutaceae. i. The mycorrhiza of *Eriostemon crowei* F.v.M. *Proc. Linn. Soc. N.S.W.* **57**: 291–312.
- MELIN, E., and NILSSON, H. (1950).—Transfer of radioactive phosphorus to pine seedlings by means of mycorrhizal hyphae. *Physiol. Plant.* **3**: 88–92.
- MORRIS, D. L., and MORRIS, CAROL T. (1939).—Glycogen in the seed of *Zea mays* (var. Golden Bantam). *J. Biol. Chem.* **130**: 535–44.
- MUELLER, F. VON (1890).—Descriptions of new Australian plants with occasional other annotations. *Vict. Nat.* **7**: 114–6.
- MUELLER, F. VON (1891).—Notes on a new Tasmanian plant of the order Burmanniaceae. *Pap. Roy. Soc. Tasm.* **1890**: 232–5.
- PEARSE, A. G. E. (1954).—“Histochemistry—Theoretical and Applied.” (J. and A. Churchill: London.)
- PFEIFFER, NORMA E. (1914).—Morphology of *Thismia americana*. *Bot. Gaz.* **57**: 122–35.
- PIJL, L. VAN DER (1934).—Die Mycorrhiza von *Burmannia* und *Epiphyllanthus* und die Fortpflanzung ihres Endophyten. *Rec. Trav. Bot. Néerl.* **31**: 761–79.
- SCHLECHTER, R. (1921).—Die Thismiae. *Notizbl. Bot. Gart. Mus. Berlin-Dahlem* **8**: 31–45.
- SWANSON, MARJORIE A. (1948).—Studies on the structure of polysaccharides. IV. Relation of the iodine colour to the structure. *Biochem. J.* **172**: 825–37.

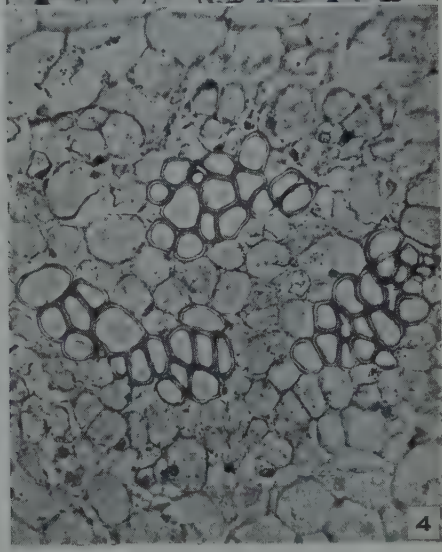
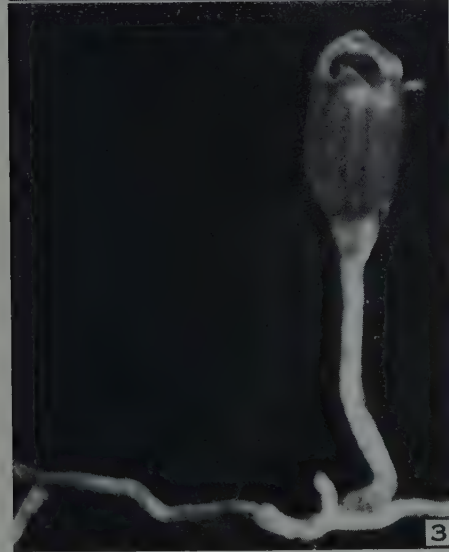
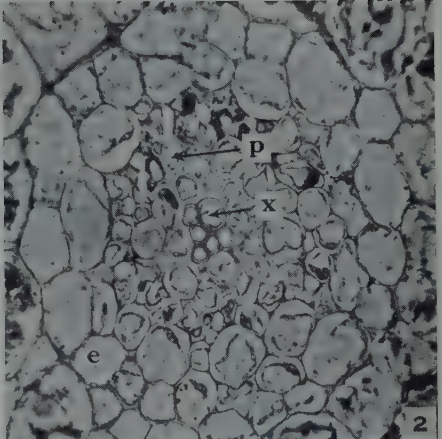
EXPLANATION OF PLATES 1–4

PLATE 1

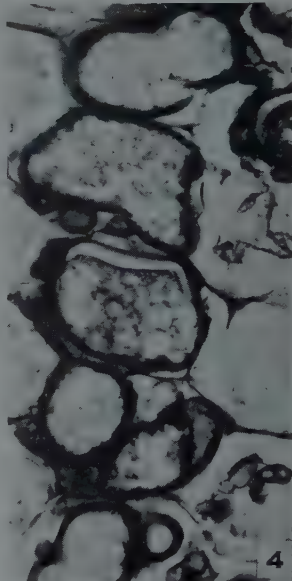
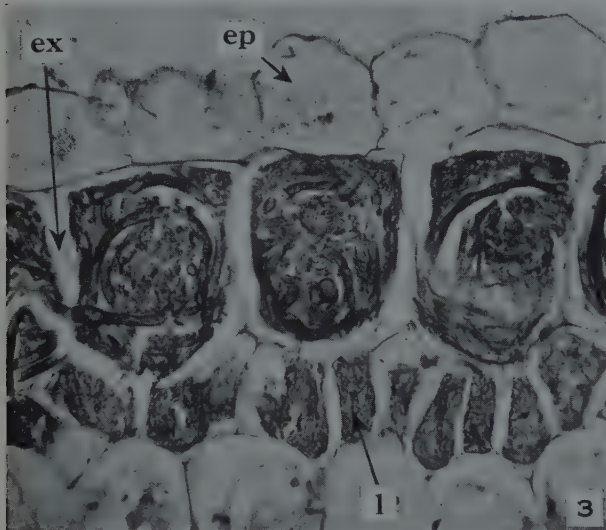
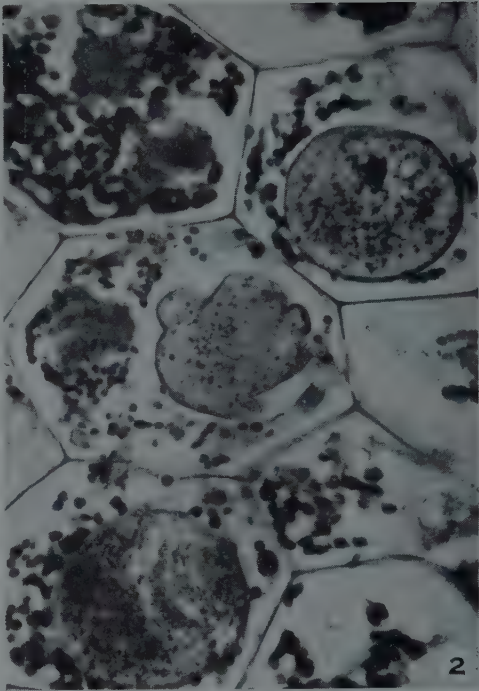
Fig. 1.—Portion of a plant of *Thismia rodwayi* with flower bud arising in the axil of a scale leaf. Natural size.

Fig. 2.—Transverse section of a root showing the stellar region. $\times 390$. *x*, xylem; *p*, phloem; *e*, endodermis.

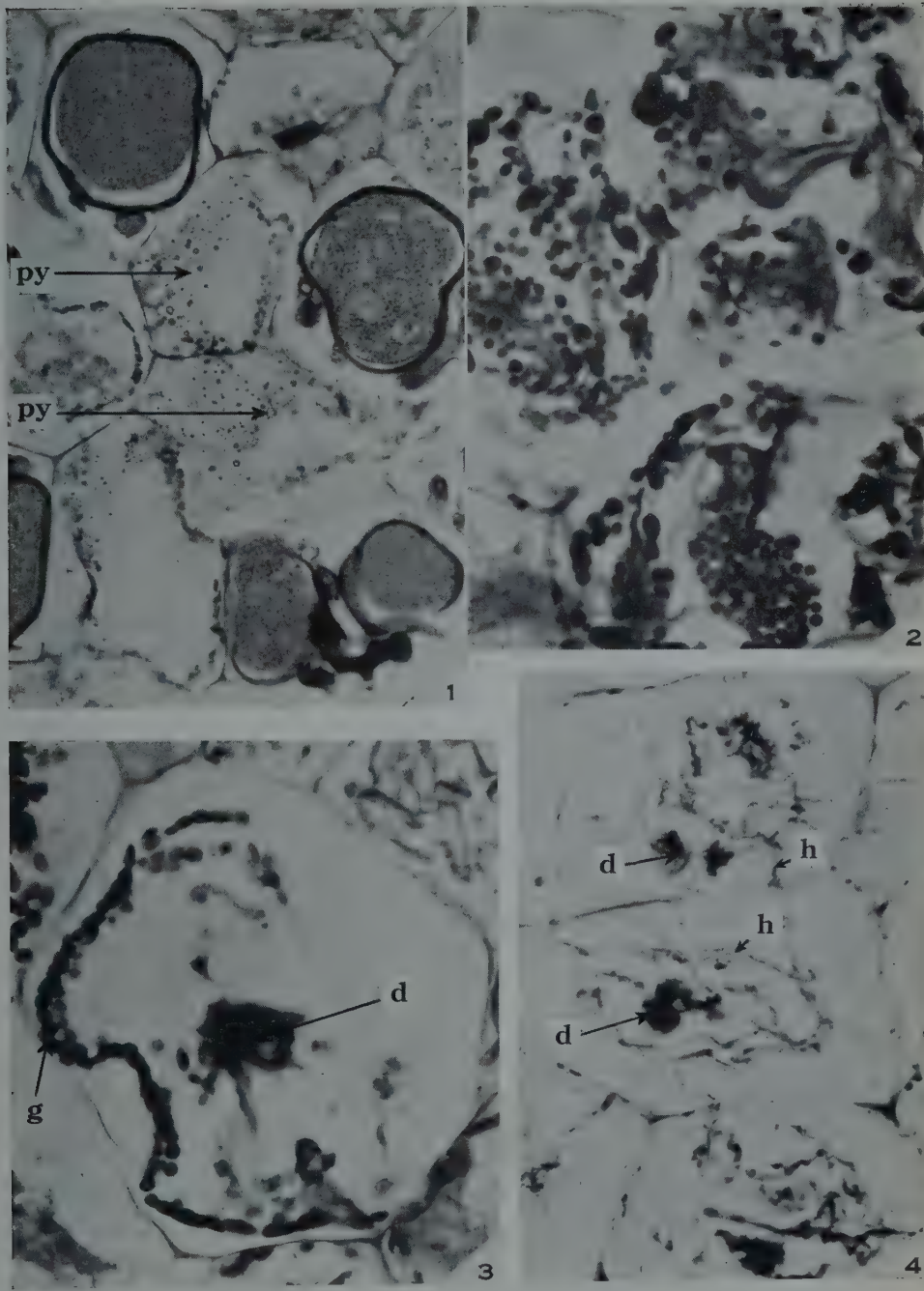
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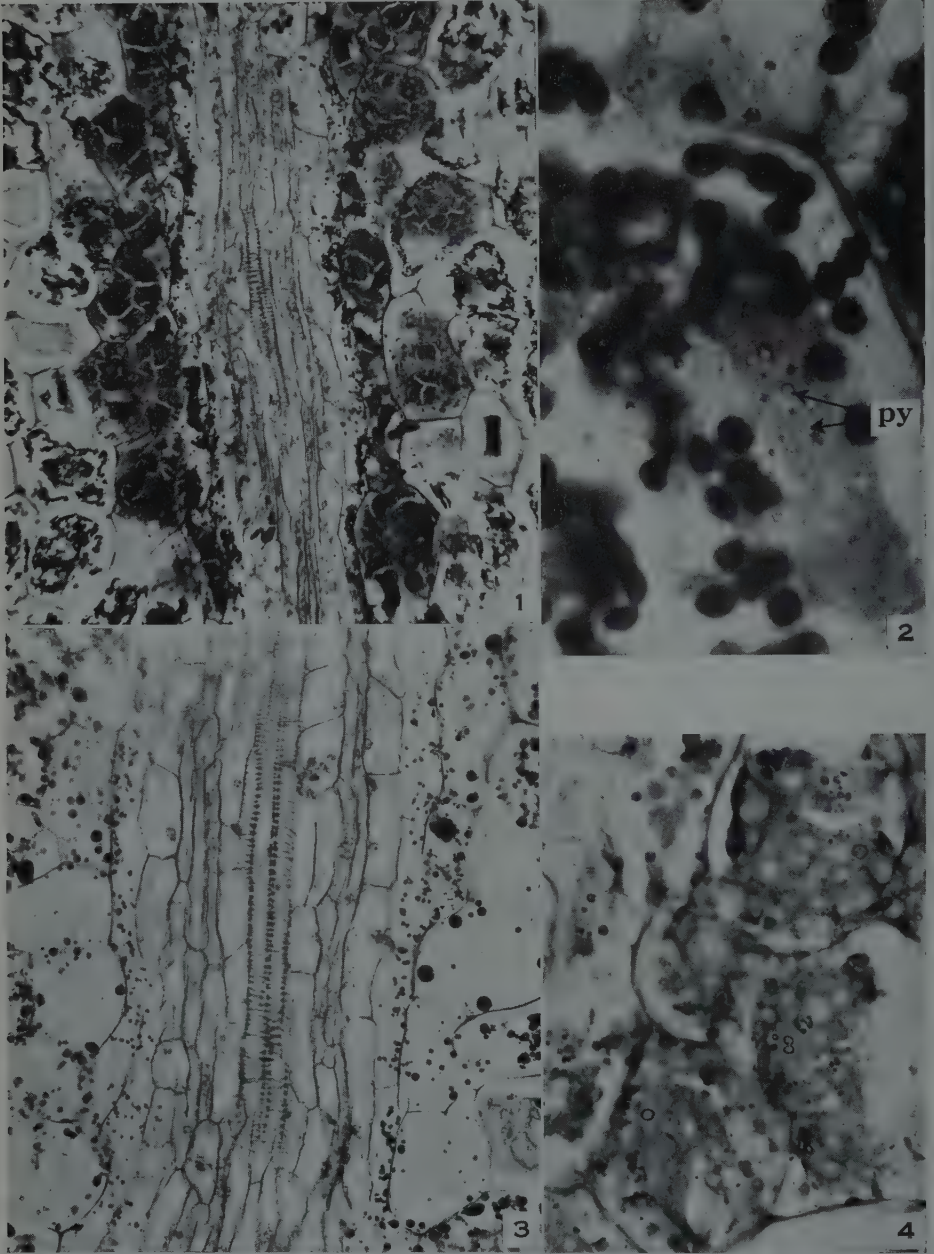


Fig. 3.—Expanded flower of *T. rodwayi* terminating a stem axis; the horizontal axis is the root. Natural size.

Fig. 4.—Transverse section of a stem. $\times 390$.

PLATE 2

Fig. 1.—Transverse section through the outer layers of the root. $\times 290$. *ep*, epidermis; *ex*, exocortex; *h*, hypha, running tangentially in the exocortical cells, giving off branches which coil in each cell.

Fig. 2.—Outer cortical cells of root to show vesicle formation. $\times 440$.

Fig. 3.—Transverse section through the exocortex and limiting layer of the root. $\times 160$. *ep*, epidermis; *ex*, exocortex containing coils of thick-walled hyphae; *l*, limiting layer with similar coils of finer hyphae.

Fig. 4.—A row of vesicles in the outer cortex cut in section. $\times 440$.

PLATE 3

Fig. 1.—Vesicles in section showing the multinucleate condition and the thick surrounding wall. $\times 450$. Note the presence of the polysaccharide material, *py*, in the neighbouring cells.

Fig. 2.—Cortical cells of the root with the globules lying in the cell lumen. The upper cell shows the remains of three digested clumps in the cell. $\times 450$.

Fig. 3.—A cortical cell of the root showing the osmic-stained globules in regular sequence in the hypha and remains of a digestive area in the cell lumen. $\times 650$. *g*, globule; *d*, digestive zone.

Fig. 4.—Cortical cells of an old root. The globules and polysaccharide deposits are no longer present in the cell. The remains of the digestive points and hyphae make up the cell contents at this stage. $\times 500$. *d*, digestive zones; *h*, hyphae.

PLATE 4

Fig. 1.—A longitudinal section through a root, stained by Best's carmine method to show the accumulation of the polysaccharide around the stele. $\times 250$.

Fig. 2.—Portion of a cortical cell of the root. $\times 1250$. A small amount of polysaccharide is present in this cell together with the osmic-stained globules. Note degradation of these globules in the mass of polysaccharide material, *py*.

Fig. 3.—A section similar to Figure 1 stained by the same method but after treatment with saliva. The treatment has removed the polysaccharide (cf. Fig. 1). A few globules are still present in the cells. $\times 250$.

Fig. 4.—Cortical cells of root, showing the replacement of the osmic-stained globules by the polysaccharide. $\times 650$. Note the dissolution of the globules as in Figure 2.

DIPLOID AND TETRAPLOID *CASUARINA LITTORALIS* SALISB. (SYN.
C. SUBEROSA OTT. & DIETR.)

By B. A. BARLOW*

[Manuscript received June 12, 1957]

Summary

Within the species *Casuarina littoralis* there are diploid and tetraploid forms, having 22 and 44 chromosomes respectively. Previous records of $2n=48$ are erroneous. The tetraploid form has an almost continuous distribution in eastern Australia and Tasmania, whilst the diploid has a small, disjunct distribution in New South Wales and Queensland. Under certain conditions hybridization has occurred.

I. INTRODUCTION

The first record of cytological work in the Casuarinaceae is that of Purcell (unpublished data†), in which *Casuarina suberosa* is quoted as having a somatic chromosome number of 48 at a few localities in Tasmania. The present author (Barlow 1955) reported that *C. suberosa* had $2n=48$ at Manly, N.S.W., although at Castle Cove, 4 miles away, the number was $2n=22$. In this latter locality the species has produced a hybrid swarm with *C. distyla*, which also has $2n=22$, and a close relationship between the two species was inferred. In the present paper the apparent aneuploid difference between the two morphologically similar forms of *C. littoralis* has been re-examined.

II. MATERIALS AND METHODS

Most of the data were obtained from a study of somatic mitoses in seedling root-tips. Pretreatment with saturated aqueous *p*-dichlorobenzene for $3\frac{1}{2}$ hr effectively shortened the chromosomes and inhibited spindle formation; fixation was carried out in acetic-alcohol and the ordinary aceto-orcein smear method was then used. Pollen mother cell meiosis was also studied in material collected from some localities, from aceto-orcein preparations.

III. OBSERVATIONAL DATA

(a) *Chromosome Numbers*

A study of the seedling progeny of a number of plants at Manly, N.S.W., showed that the chromosome number is actually $2n=44$. The author's previous determination of $2n=48$, which conformed with Purcell's results in Tasmania, is probably incorrect. The correction has resulted from the use of *p*-dichlorobenzene as a chromosome shortening agent. The unshortened chromosomes are fairly small, they vary considerably in length, and they have a number of secondary constrictions, so that delimitation of individual chromosomes is difficult. A longer chromosome with a large constriction could be mistaken for two. The use of the shortening agent has eliminated these difficulties (Plate 1, Fig. 1).

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†Purcell is incorrectly listed by Darlington and Wylie (1955), as having published this work in *Pap. Roy. Soc. Tasm.* in 1953.

Samples were collected from many localities, including Tasmania, with the aims of checking Purcell's records and of determining the distributions of the chromosome forms within the species. Chromosome numbers for each locality were derived from three to eight counts (mean five) on the progeny of one or two plants. The results are illustrated in Figures 1 and 2.

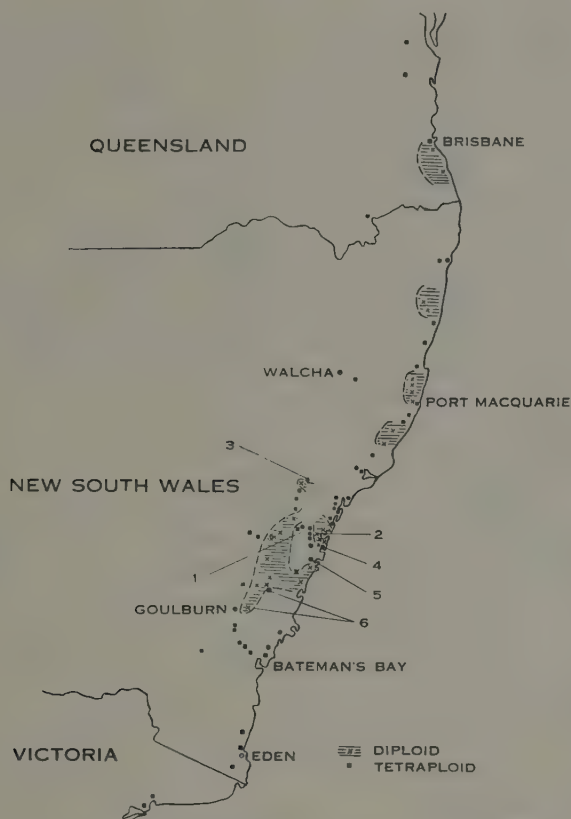


Fig. 1.—Distribution of diploid and tetraploid *Casuarina littoralis*.
See Section III(b) for explanation of numbers 1–6.

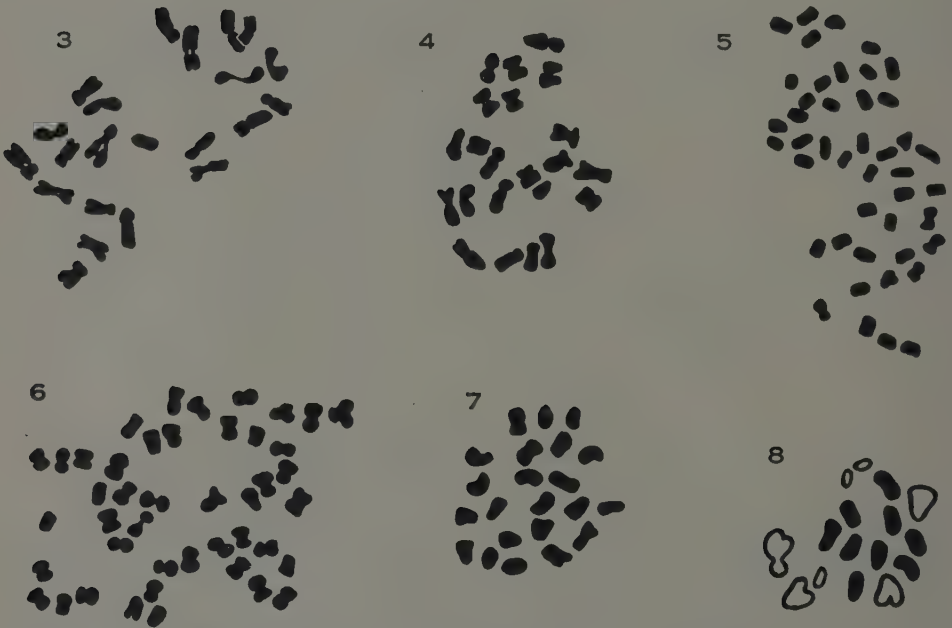
Whilst the localities studied do not provide an even cover of the whole range of *Casuarina littoralis* they are sufficient to allow some inferences to be made about the variation of chromosome number within the species.

It is probable that there are only two chromosome forms, diploid and tetraploid, with somatic numbers of 22 and 44 respectively (Figs. 3–7; Plate 1, Figs. 1 and 2). The tetraploid form extends almost throughout the species range in eastern Queensland, New South Wales, Victoria, and Tasmania. The diploid form occurs in several apparently disjunct regions. There are two in the Sydney district, one of which has an extension into the central and near southern highlands of New South Wales. However, the apparent discontinuities in the diploid range in northern New South

Wales and southern Queensland require confirmation. The boundaries marked around the localities of the diploid form must be regarded as purely tentative; they



Fig. 2.—Distribution of tetraploid *Casuarina littoralis* in Tasmania.



Figs. 3-8.—*Casuarina littoralis*. Figs. 3-6, somatic mitosis in root tips. All treated with *p*-dichlorobenzene. Fig. 3.—Muogamarra, $2n=22$. Fig. 4.—Burraborang Valley, $2n=22$. Fig. 5.—28 miles N. of Bateman's Bay, $2n=44$. Fig. 6.—10 miles NW. of Hobart, $2n=44$. Fig. 7.—P. M. C. meiosis. Manly, M I, $n=22$. Fig. 8.—P. M. C. meiosis. Triploid hybrid, Manly, M I, with trivalents and univalents in outline. All $\times 2,500$ approx.

have been inserted to show that the diploid and tetraploid forms can be separated geographically.

(b) Exceptional Cases

From a few localities chromosome numbers other than 22 or 44 have been recorded. In some of these cases hybridization between the two forms has probably occurred. The localities have been numbered 1-6 in Figure 1; details of the findings are as follows:

| | | |
|---|--------------------------|--|
| 1 | 12 miles N. of Glenorie | One count of 66 (all sibs 44) |
| 2 | Muogamarra | One count of 33 (all sibs 22) |
| 3 | 30 miles S. of Singleton | Three counts of 44 and seven of 33 from seedlings of one plant |
| 4 | Manly | } Apparent hybridization—see below. |
| 5 | Oatley | |
| 6 | Goulburn District | |

The origin of the somatic number of 66 in a seedling from Glenorie is not clear. Since sib seedlings had $2n=44$, it had most probably arisen from non-reduction of a gamete. At Muogamarra, all other counts were diploid ($2n=22$), and a similar origin of the somatic number 33 may have occurred. At other localities, hybridization between the diploid and tetraploid forms is probable, since they are close to boundaries between the two forms.

(c) Morphology

Morphologically the two forms are very similar. In the northern parts of its range the diploid form can usually be distinguished from the tetraploid by its somewhat more slender foliage and its smaller, more compact cones with more sharply pointed valves. However, the diploid form in the south is coarser in habit than it is in the north, and overlaps the tetraploid in branchlet diameter and length, and in internodal length, so that there is at present no certain way of distinguishing the two forms in the field. It is possible that, as a result of a more detailed inquiry, constant differences between the two forms may be recognized.

(d) Hybridization

Hybridization is apparent in places where the two forms are in contact. The degree of interbreeding varies from one locality to another, and is probably correlated with the extent of human disturbance at each site (Cain 1944; Barlow 1955).

(i) *Goulburn-Mittagong District*.—Somatic chromosome numbers of the seedling progeny of 26 trees growing in the Goulburn-Mittagong district were counted (Table 1). Both diploid and tetraploid forms occur in the district. Most batches of seed showed complete uniformity in chromosome number, being all diploid or all tetraploid; but four groups of sibs provide evidence for hybridization. In three of these, some triploids were found, the sibs being either diploid (Mittagong) or tetraploid (Bowral). Such occurrences are reasonably explained as the immediate products of hybridization between the two forms. In the fourth sib progeny (15 miles SW. of Berrima) chromosome numbers varied from 25 to 32. The parent tree is probably a triploid hybrid, and the progeny the result of backcrossing to the diploid.

(ii) *Manly*.—Most of the trees at Manly are tetraploids with $2n=44$. From the somatic mitoses in shoots it has been found that at least one diploid plant is present. Besides the apparently rare diploid there is also a population of *C. distyla* ($2n=22$) occupying adjacent sandstone ridges.

TABLE 1
CHROMOSOME NUMBERS OF SEEDLING PROGENIES FROM GOULBURN-MITTAGONG DISTRICT

| Locality | Sample | Number of Seedlings with Somatic Numbers | | | Other Chromosome Numbers |
|------------------------------|--------|--|----|----|---------------------------|
| | | 22 | 33 | 44 | |
| 3½–4½ miles NE. of Mittagong | 1 | 6 | | | |
| | 2 | 5 | | | |
| | 3 | 4 | | | |
| | 4 | 7 | | | |
| | 5 | 5 | | | |
| | 6 | 5 | | | |
| | 7 | 7 | | | |
| | 8 | 2 | | | |
| | 9 | 3 | | | |
| | 10 | 4 | | | |
| Mittagong | 1 | 10 | 1 | | |
| | 2 | 4 | 1 | | |
| 2 miles W. of Mittagong | 1 | | | 2 | |
| 16 miles W. of Mittagong | 1 | 4 | | | |
| Wombeyan Caves | 1 | | | 4 | |
| Bowral | 1 | | | 2 | |
| | 2 | | 2 | 5 | |
| 1 mile NE. of Berrima | 1 | | | 3 | |
| 15 miles SW. of Berrima | 1 | | | | 25, 28, 28, 29, c. 30, 32 |
| | 2 | 4 | | | |
| 17 miles SW. of Berrima | 1 | | | 4 | |
| | 2 | | | 4 | |
| 23 miles NE. of Goulburn | 1 | 4 | | | |
| | 2 | 4 | | | |
| 15 miles E. of Goulburn | 1 | 1 | | | |
| | 2 | 5 | | | |

A number of plants are morphologically intermediate between *C. littoralis* and *C. distyla*, and are presumably hybrids (Barlow 1955). Pollen mother cell meiosis is

highly erratic and fertility is low, as would be expected in triploid hybrids. Multivalents and univalents occur frequently and at Anaphase I lagging chromosomes are common and sometimes show misdivision. The chromosome material which is excluded from the first and second division spindles usually gives rise to microcytes, so that polysporous tetrads are frequent.

The same cytological behaviour occurs in triploid plants which are morphologically true *C. littoralis*, and it is assumed that these are hybrids between the diploid and tetraploid forms of this species (Fig. 8). The products of meiosis range from diads (through restitution) to octads (see Table 2), and the frequency of apparently functional pollen is less than 20 per cent.

TABLE 2
FREQUENCY OF MICROCYTES AFTER MEIOSIS IN HYBRIDS, AT MANLY

| Plant | Diads | Triads | Tetrads | Pentads | Hexads | Heptads | Octads |
|-------|-------|--------|---------|---------|--------|---------|--------|
| 1 | 2 | 2 | 40 | 45 | 24 | 8 | 6 |
| 2 | 1 | 3 | 31 | 49 | 12 | 3 | 1 |

One plant which was found to have 37 chromosomes at meiosis is probably a backcross to the tetraploid. The divisions are unbalanced, and microcytes are formed, although the meiosis is generally more regular than in the triploids.

(iii) *Oatley*.—Most of the seedling progeny examined from Oatley had 44 chromosomes, but some were aneuploid, having from 39 to 43 chromosomes (Plate 1, Fig. 3). Most of the male plants had regular meiosis and were highly fertile, but one of the plants examined had *c.* 38–40 chromosomes, and an irregular meiosis, with the formation of microcytes in 20 per cent. of the pollen mother cells.

The unbalance in this population may be a result of hybridization with the diploid form, as it is known to occur nearby at National Park.

(e) Seedling Growth Rates

Batches of diploid and tetraploid seedlings were grown from seed collected at Appin, where both forms occur in one population. They were grown side by side in tubes at the University of Sydney and later transplanted to an experimental farm at Castle Hill.

Morphologically the diploid and tetraploid seedlings are indistinguishable, but in the early seedling stages the tetraploids have a distinctly more rapid growth rate than the diploids (Plate 2). The growth rates of the two forms are compared in Table 3. The more vigorous shoot development of the tetraploid is correlated with stronger root development.

As the seedlings grow older, the size difference between the diploids and tetraploids diminishes until, at the age of $1\frac{1}{2}$ years, they are about the same size.

Nevertheless, a more vigorous growth in the seedling stage, which is the most critical in the life history, may give the tetraploid form a distinct competitive advantage over the diploid.

TABLE 3
MEAN SHOOT LENGTH OF SEEDLING PROGENIES FROM APPIN (ABOUT 6 MONTHS OLD)

| Parent Plant Number | Chromosome Number | Number of Seedlings Observed | Mean Epicotyl Length (cm) |
|---------------------|-------------------|------------------------------|---------------------------|
| 102.02 | 22 | 12 | 6.0 \pm 0.3 |
| 102.04 | 22 | 36 | 7.2 \pm 0.4 |
| 102.07 | 22 | 27 | 6.7 \pm 0.3 |
| 102.16 | 44 | 13 | 11.6 \pm 0.3 |
| 102.20 | 44 | 3 | 10.1 \pm 0.4 |

IV. DISCUSSION

The results indicate that there are two cytological forms of *Casuarina littoralis*: a tetraploid form with an extensive range from Tasmania to Queensland, and a diploid form with a range of 400 miles or more in the coastal districts of New South Wales and Queensland. The disjunct distribution of the diploid suggests that it has previously occupied a wider range than at present, and its range has been much dissected, possibly as a result of competition with the tetraploid form. This theory is supported by the observation that tetraploid seedlings have a more vigorous growth than diploids.

The wide range of the tetraploid form suggests that it is relatively ancient in origin. It has probably spread well beyond the geographical limits of the original diploid population, into Victoria and Tasmania, where the diploid form has not been recorded. The tetraploid form is apparently more "aggressive" than its diploid progenitor (cf. Stebbins 1950).

Evidence for hybridization has only been found in places where the two forms actually come into contact. Apparently the hybrids are relatively localized, even though the species is wind-pollinated. At points of hybridization, the frequency of the hybrids varies, but in most cases they form only a small fraction of the total number of plants. This, together with the reduced fertility of the hybrids, means that there is only a limited amount of gene flow from one form to the other as a result of introgression, so that the two forms are effectively isolated.

Although there is little doubt that the tetraploid race is ancient in origin, there is no evidence to show whether it has had a single or a multiple origin. The absence of multivalents in the tetraploid form suggests strongly that it has had an allopolyploid origin, or else a very ancient autopolyploidy with sufficient time for "rediploidization". The fact that the two forms are morphologically similar does not preclude a hybrid origin for the tetraploid; a number of examples are known in which an allotetraploid is indistinguishable from one or other of its diploid parents

(Howard and Manton 1940, 1946; Clausen, Keck, and Hiesey 1945; Uhl 1952). There are a number of forms with 22 chromosomes in the "*distyla* complex", to which *C. littoralis* is closely related, and it has already been shown that diploid *C. littoralis* produces fertile hybrids with one member of this group.

The suggestion that the tetraploid race is more vigorous than the diploid also conforms with the idea that it may have had a hybrid origin. In a number of grasses it has been shown that autotetraploid is weak and inferior in competition with its diploid progenitors (Stebbins 1949; Sakai and Suzuki 1955*a*). On the other hand, allopolyploids are often more vigorous than their diploid parents, and have a higher competitive ability (Stebbins 1947). This has been shown with interspecific crosses in *Nicotiana*, and with *Triticale*, which is the result of an intergeneric cross (Sakai and Suzuki 1955*b*). Many species and forms which were previously considered to be of autopolyploid origin are now thought to have arisen from intervarietal or interspecific crosses (Stebbins 1947). On the basis of the present knowledge of the two forms of *Casuarina littoralis* the theory of a hybrid origin of the tetraploid is quite consistent with conditions known to occur in other species of flowering plants.

V. CONCLUSIONS

(i) Within the species *Casuarina littoralis* there are diploid and tetraploid forms with 22 and 44 chromosomes respectively.

(ii) Previous reports that *C. littoralis* has 48 chromosomes are probably incorrect.

(iii) The tetraploid race is more aggressive than the diploid, and is much more widely distributed. It has probably extended the range of the species. The diploid has a disjunct and relic distribution.

(iv) Under certain conditions the two races have hybridized.

(v) It is probable that the tetraploid race is allotetraploid, having arisen from the diploid through hybridization.

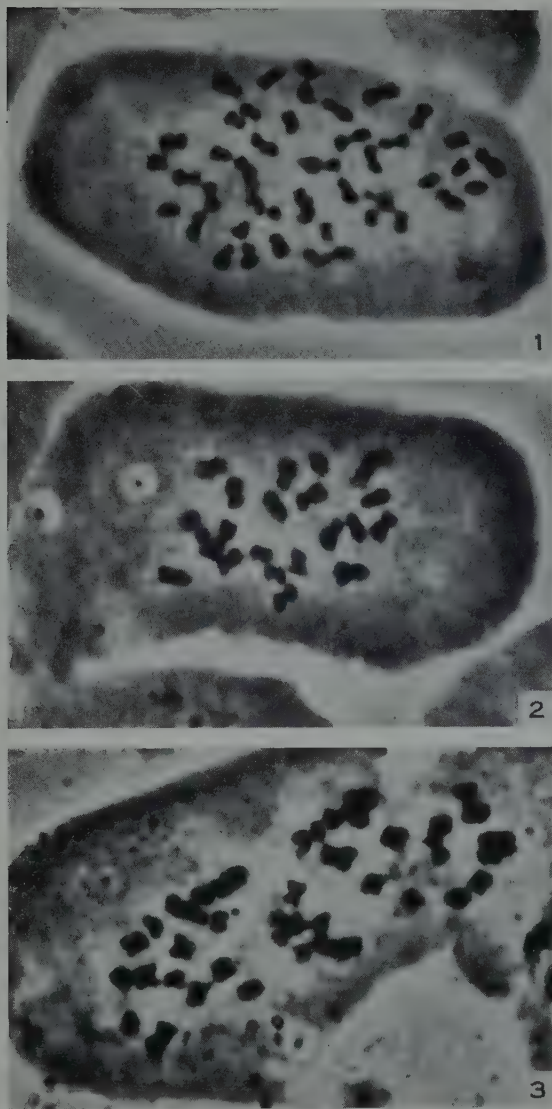
VI. ACKNOWLEDGMENTS

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VII. REFERENCES

- BARLOW, B. (1955).—A new chromosome form of *Casuarina suberosa*. *Proc. Linn. Soc. N.S.W.* **80**: 285.
- CAIN, S. A. (1944).—"Foundations of Plant Geography." (Harper and Bros.: New York.)
- CLAUSEN, J., KECK, D. D., and HIESEY, W. M. (1945).—Experimental studies on the nature of species. II. Plant evolution through amphiploidy and autopolloidy, with examples from the Madiinae. Carnegie Inst. Washington, Publ. No. 564.
- DARLINGTON, C. D., and WYLIE, A. P. (1955).—"Chromosome Atlas of Flowering Plants." 2nd Ed. p. 182. (Allen and Unwin : London.)
- HOWARD, H. W., and MANTON, I. (1940).—Allopolyploid nature of the wild tetraploid watercress. *Nature* **146**: 303.

- HOWARD, H. W., and MANTON, I. (1946).—Allopolyploid and autopolyploid watercress (*Nasturtium*) with the description of a new species. *Ann. Bot.* (new ser.) **10**: 1–13.
- SAKAI, K.-I., and SUZUKI, Y. (1955a).—Studies on competition in plants. II. Competition between diploid and autotetraploid plants of barley. *J. Genet.* **53**: 11–20.
- SAKAI, K.-I., and SUZUKI, Y. (1955b).—Studies on competition in plants. V. Competition between allopolyploids and their diploid parents. *J. Genet.* **53**: 585–90.
- STEBBINS, G. L. (1947).—Types of polyploids: their classification and significance. *Advanc. Genet.* **1**: 403–29.
- STEBBINS, G. L. (1949).—The evolutionary significance of natural and artificial polyploids in the family Gramineae. *Proc. 8th Int. Congr. Genet. (Hereditas, Suppl. Vol.)*, pp. 461–85.
- STEBBINS, G. L. (1950).—“Variation and Evolution in Plants.” (Columbia Univ. Press.: New York.)
- UHL, C. H. (1952).—Heteroploidy in *Sedum rosea*. *Evolution* **6**: 81–6.

DIPLOID AND TERTAPLOID *CASUARINA LITTORALIS*

Mitosis in root tips of *Casuarina littoralis* seedlings. All treated with *p*-dichlorobenzene. $\times 2200$.

Fig. 1.—Manly, $2n=44$.

Fig. 2.—14 miles E. of Goulburn, $2n=22$.

Fig. 3.—Oatley, 38 chromosomes.

DIPLOID AND TETRAPLOID *CASUARINA LITTORALIS*

Seedling progeny of *Casuarina littoralis* from Appin, at 6 months old. 102-02, 102-04, and 102-07 are diploids; 102-16 and 102-20 are tetraploids. $\times 0.55$.

THE EFFECT OF QUADRAT SIZE, PLANT SIZE, AND PLANT DISTRIBUTION ON FREQUENCY ESTIMATES IN PLANT ECOLOGY

By J. E. C. ABERDEEN*

[*Manuscript received August 29, 1957*]

Summary

The theoretical foundation for frequency estimates, as used in plant ecology, is discussed. An equation is derived in which the absence value is linked with the quadrat size, the plant unit size, the plant density, and the aggregation of the plant units.

Graphical methods are used to estimate the density and the average size of the individuals of a species for a random distribution. Departure from a random distribution can also be detected by these methods.

It is shown that if an estimate of the average plant unit size is combined with the frequency estimates, then the reliability of the results is increased considerably.

The value of frequency estimates made with one, two, or more sizes of quadrats is discussed.

I. INTRODUCTION

The application of frequency estimates to the quantitative analysis of plant communities has been briefly, but clearly, outlined by Brown (1954). The method is commonly used to give a relative estimate of the importance of each species in a community, but if the sampling procedure has been satisfactory, and the individual plants of a species are distributed at random, then the absolute density of the species can be calculated.

While the value obtained is simply called "the frequency", it is actually a percentage figure. It is derived by selecting at random, within the area to be examined, a number of sample areas or quadrats of fixed size, and recording each plant species as present in, or absent from, each quadrat. For each species, the frequency equals the percentage of quadrats in which it occurs. The method has been widely used in plant ecology, but the lack of understanding of the conditions underlying it has led, in some cases, to a false picture of the plant community under consideration. The defects have been pointed out by Blackman (1935) and Ashby (1935), among others, and more recently by Goodall (1952*a*). Preston (1948) and Williams (1950) have discussed the relationship of these values to particular theoretical populations. Goodall (1952*a*) summarizes the difficulties peculiar to frequency estimates as follows: "Certainly the frequency found reflects certain absolute characteristics of the vegetation, as well as the size and distribution of the quadrats used; but it combines so many (density, distribution, and in many cases size of individuals), and unites them in so complex a fashion, that it is not usually possible to argue back from the frequency to the features of the vegetation on which it depends."

Another possible pitfall in the use of frequency percentages was demonstrated by Williams (1950), who investigated their use for comparing the compositions of different plant communities. He showed that frequency percentages were not

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comparable unless the areas of the quadrats used were adjusted proportionally to a particular basic characteristic of each community, i.e. the index of diversity number.

In most instances where frequency has been used to indicate the relative importance of a species, it has been interpreted as a relative estimate of either density or plant cover. The techniques used in these circumstances fall into two groups. More frequently the size of the quadrat is large, relative to the size of the plant unit. Then the plant is recorded as present in either of two ways: (i) if any part of it falls within the quadrat, or (ii) only if its centre falls within the quadrat. Method (i) is unsound if no allowance is made for plant size; any reasonable success achieved by using it is most probably due to the large size of the quadrat relative to the plant unit, or to a low density of the plant units. Less frequently, a quadrat of point dimensions (Goodall 1952*b*) is used. In its simplest form a large needle is placed vertically amongst the community at randomly selected points. The percentage of times that each species touches the needle is then recorded. Consideration of these two techniques shows that if the density of the species is constant, then the frequency estimates will vary with the size of the quadrat in the first group of techniques, and with the size of the individual plant unit if the point quadrat method is used. Point quadrats are, however, normally employed to estimate total coverage with no attempt to separate the effects of plant unit size and density.

Hoell (1943) made allowance for plant size when he used both quadrat and transect methods of sampling to investigate the relative efficiency of estimates of frequency, coverage, and abundance. He emphasized the relative inefficiency of frequencies. This is generally recognized, however, and is inherent in the method, whose chief value lies in the saving of time and labour. Archibald (1952) investigated empirically the relative effect of plant unit size on frequencies. She used varying sizes of quadrats on a model of a plant community drawn on a large sheet of graph paper, and then compared her results with those obtained in natural communities. A plant was marked present if even the smallest part was enclosed within the quadrat. She thus showed that the average size of the individual plants of any one species did exert an appreciable effect on the estimate of frequency. She suggested that the results could be used to estimate total cover, or basal areas, for the various species, but she did not suggest how the two effects of quadrat size, and plant unit size, could be separated, nor did she give any mathematical basis to the work. Aberdeen (1954) has shown how quadrat size, plant unit size, density of plants, and frequency values can be brought together in a single equation, assuming a random distribution of the plants and the quadrats. The equation derived there is as follows:

$$a = e^{-\pi(R+r)^2d}, \dots\dots\dots(1)$$

where a is the proportion of quadrats from which the species is missing; R , the radius of a circular quadrat; r , the radius of a circle covered by a plant unit; and d , the density of the plant units.

Archibald's results are particularly suitable for checking the validity of equation (1). They also provide a clue to a further step in the use of frequencies, i.e. for the detection of non-random distributions. The following section of this paper investigates these two aspects.

II. METHODS AND RESULTS

Archibald (1948) has already investigated some of her results to ascertain whether individuals of the species were randomly distributed or not. To do this she used the counts of numbers of individuals per quadrat, and Neyman's equation for a contagious distribution. The methods to be considered here use only the frequency percentage and the corresponding quadrat size. It should be noted that frequency and absence are expressed as proportions in the following equations and graphs, i.e. 100 per cent. = 1.00.

For the purpose in hand, equation (1) is expressed in a slightly different form, viz.

$$\log a = -\pi(R+r)^2d \log e. \quad \dots\dots\dots(2)$$

Therefore

$$\sqrt{-\log a} = k(R+r), \quad \dots\dots\dots(3)$$

where

$$k = \sqrt{(\pi d \log e)},$$

and d is constant. Equation (3) would thus be applicable to a series of frequency estimates from the same population, using quadrats of varying sizes.

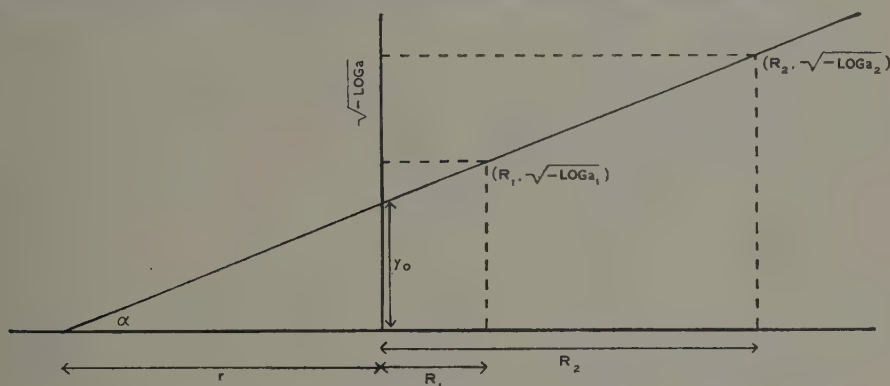


Fig. 1.—Diagram showing relationship between absence values and quadrat radius.

From equation (2), if the size of the unit itself is neglected it follows that

$$-\log a \propto d,$$

for a single size of quadrat. This is the relationship first used by Blackman (1935). If R is negligible, i.e. point quadrats are used, then

$$-\log a = A \log e,$$

where A is equal to $\pi r^2 d$, i.e. the proportion of the total area covered by the plant units, where there is no overlapping of individual units.

From equation (3) a simple graphical method can be deduced. The relationships are shown in Figure 1, where corresponding values of $\sqrt{-\log a}$ are plotted against two quadrat sizes, R_1 and R_2 . Thus by plotting the values for $\sqrt{-\log a}$ against the known corresponding quadrat radii, and continuing the regression line until it cuts the x -axis, it is seen that the latter intercept will equal $-r$, where r is the average diameter of the plant unit of the species under consideration.

Figure 2 has been prepared to enable the reader to visualize the relationship between frequency and $\sqrt{-\log a}$. It will be noticed that the relationship closely approximates a straight line for frequency values from 0.05 to 0.90.

The density of the individuals of a species can also be calculated. From equation (2)

$$\begin{aligned} d &= \frac{-\log a}{\pi (R+r)^2 \log e}, \\ &= \frac{1}{\pi \log e} \times \frac{-\log a}{(R+r)^2}. \end{aligned}$$

From the relationships of Figure 1 then

$$d = \frac{1}{\pi \log e} \times \tan^2 \alpha, \quad \dots \dots \dots (4)$$

where α is the angle that the regression line makes with the x -axis.

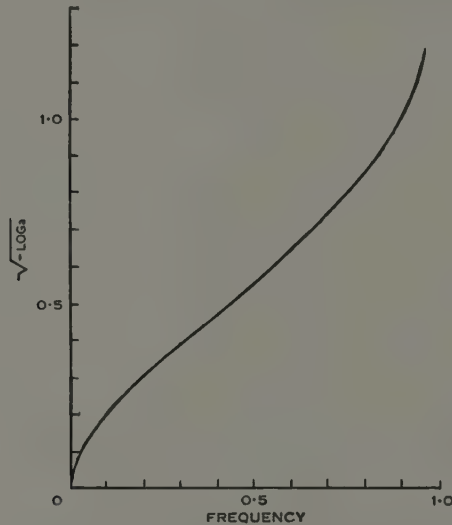


Fig. 2.—Diagram illustrating the relationship between frequency (as a proportion) and $\sqrt{-\log a}$.

In any random distribution a number of the units will overlap others. The actual area occupied by the species can be calculated as follows:

Let P_0 = the proportion of the area actually occupied by the plant units,

a_0 = the absence value when $R = 0$, and

y_0 = the value of the intercept cut off by the regression line on the y -axis.

If a point quadrat is used, i.e. $R = 0$, then

$$P_0 = 1 - a_0,$$

$$y_0 = \sqrt{-\log a_0},$$

therefore

$$P_0 = 1 - 10^{-y_0^2}. \quad \dots \dots \dots (5)$$

Thus estimates are obtained for (i) the average radius, (ii) the average density, and (iii) the area covered, for the species under consideration.

The data collected by Archibald (1952) were then used to check these equations. In her model layout, in addition to listing the frequency percentages and the sizes of quadrats used, she also recorded the original unit size, and total area covered, for each of her "species". From the last two values, the radii and the densities of the

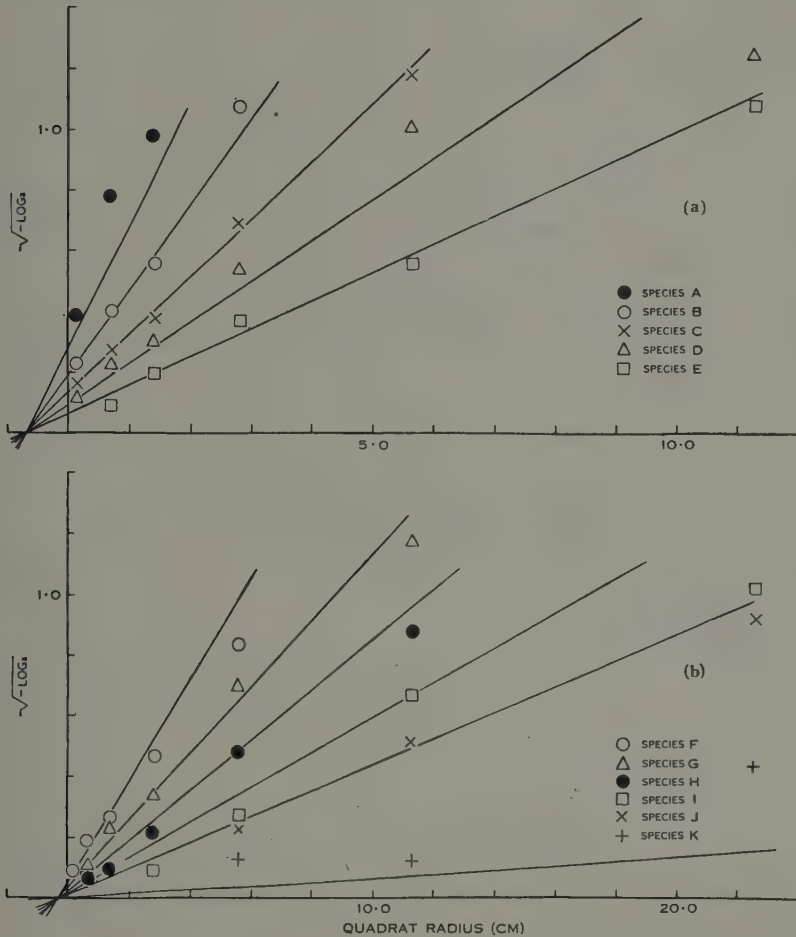


Fig. 3.—Graph demonstrating the relationship between $\sqrt{-\log \alpha}$ and quadrat radius using data from Archibald (1952). The solid lines, derived from given data, are the theoretical expected regression lines. The points indicated by the symbols are from experimental observations. (a) species A–E, (b) species F–K.

units can be calculated. For the purpose of the calculations, both the quadrats and the species units have been treated as circles, with areas equal to the squares used in her paper. Then, using equation (4), the angle α was calculated for each species. Alternatively, from the proportion of the area covered, using equation (5), the intercept y_0 can be calculated. Using the known radius of the species, and one of these two previous values, the regression line for each species has been drawn. These are shown in Figure 3 as solid lines.

TABLE I
COMPARISON OF SPECIES CHARACTERISTICS AS CALCULATED FROM KNOWN DATA AND OBSERVATIONS FROM TRIALS

| Species | Unit Radius* (cm) | | Area Covered (Proportion) | | Density (Units per sq. cm.) | |
|---------|-----------------------|---------------------|------------------------------|---------------------|--------------------------------|---------------------------------------|
| | | | | | | |
| | From Known Data | From Observation | From Known Data | From Observation | From Known Data | From Observations Using eqn.(4) |
| | | | | | Area Covered/ Area of Unit | Using eqn.(4)† |
| A | 0.70 | 0.83 | 0.17 | 0.28 | 0.103 | 0.122 |
| B | 0.70 | 0.57 | 0.083 | 0.070 | 0.052 | 0.056 |
| C | 0.70 | 0.82 | 0.042 | 0.047 | 0.026 | 0.028 |
| D | 0.70 | 1.85 | 0.021 | 0.079 | 0.013 | 0.014 |
| E | 0.70 | 0.77 | 0.010 | 0.011 | 0.0065 | 0.0065 |
| F | 0.28 | 0.50 | 0.0052 | 0.011 | 0.020 | 0.021 |
| G | 0.28 | 0.78 | 0.0026 | 0.013 | 0.010 | 0.010 |
| H | 0.28 | 0.077 | 0.0013 | 0.0010 | 0.0050 | 0.0052 |
| I | 0.28 | 0.42 | 0.00065 | 0.00090 | 0.0025 | 0.0026 |
| J | 0.28 | 0.74 | 0.00032 | 0.0020 | 0.0013 | 0.0013 |
| K | 0.14 | 0.00 | 0.0000016 | 0.00080 | 0.000024 | 0.00026 |

*The unit radius is the radius of a circle having the same area as the corresponding species unit of Archibald (1952).

†Density of units as estimated from this equation will be greater than that obtained by multiplying the proportion of area covered by the size of unit, as it makes allowance for the overlapping of units which occurs in a random distribution.

The values for $\sqrt{-\log a}$, independently obtained from her experimentally observed frequency percentages for the various sizes of quadrats, have been used to plot a series of points for each of the species. The overall agreement between these points and the calculated lines is satisfactory. The values for species D suggest a curve, but this is due to only one value, that for a quadrat of radius 11.3 cm. The others show satisfactory straight-line relationships, with slight differences in slopes for species A, F, H, I, and K. Species K shows the most irregularity. The departures from the calculated lines do not show any consistent directional bias, so presumably they are due to chance.

In Table 1 the approximate values for the radii, the proportions of the total area covered, and the densities, as calculated from the known data, are compared with those estimated from the observed frequencies. The latter were obtained from regression lines fitted by eye to each set of plotted points.

Following this the data from the field surveys as used by Archibald (1952) were investigated. Figures 4(a) and 4(b) were obtained by plotting $\sqrt{-\log a}$ against the quadrat size for selected species from the three communities, viz. *Salicornia* marsh, *Limonium* marsh, and *Eriophorum* bog. The curves were fitted by eye to the plotted points. The striking difference from the results for the model layout is the curvature of some of the lines, particularly those for *Festuca* sp. and *Salicornia stricta*.

Archibald (1948) had already investigated the distribution of a number of the species from these communities using Neyman's contagious distribution equation. *Festuca* sp. and *Salicornia stricta* (*Limonium* marsh) were shown there to have a contagious distribution. Of the remaining species *Spergularia marginata* and *Limonium vulgare* were shown to be random, but in communities other than those in which they occur for Figures 4(a) and 4(b). *Aster tripolium* was shown to be random at one time of the year, but non-random later in the season. The other three species were not discussed in that paper.

Considering now the remainder of data in Figures 4(a) and 4(b) the regression lines for *Webera nutans*, *Aster tripolium*, and *Spergularia marginata* are seen to be straight, indicating a random distribution. The remaining species, viz. *Calluna vulgaris*, *Lepturus* sp., and *Limonium vulgare* would probably show no significant departure from a straight-line relationship, but a relationship which allowed for a sudden dip as the quadrat size approached zero would very likely give a closer fit. This is due entirely to the zero frequencies for the small quadrats. In the light of Archibald's (1948) results, the most likely explanation of the curvature of lines in Figures 4(a) and 4(b) is a contagious distribution.

This possibility of a contagious distribution is considered by extending the theory used to develop equation (1). It is assumed that the plants are gathered together in groups, and that the area covered by each group is in the form of a circle. Within each circle the plants are assumed to be distributed at random. These circular groups of plants will be referred to as "aggregation circles". The term "cluster" has been deliberately avoided to prevent confusion with Archibald's paper, where it is equivalent to a plant unit.

For purposes of the following discussion, let r_1 be the average radius of the plant units; d_1 , the average density of the plant units within any one aggregation

circle; r_2 , the average radius of the aggregation circle, the enclosing circle being determined by the outer points of the two or three outlying units; and d_2 , the average density of occurrence of the aggregation circles. From equation (1), the proportion of the entire area occupied by the aggregation circles is $1 - \exp(-\pi r_2^2 d_2)$.^{*} This value is the equivalent of the probability that a point taken at random would fall within an aggregation circle. Then the probability of a point quadrat not contacting

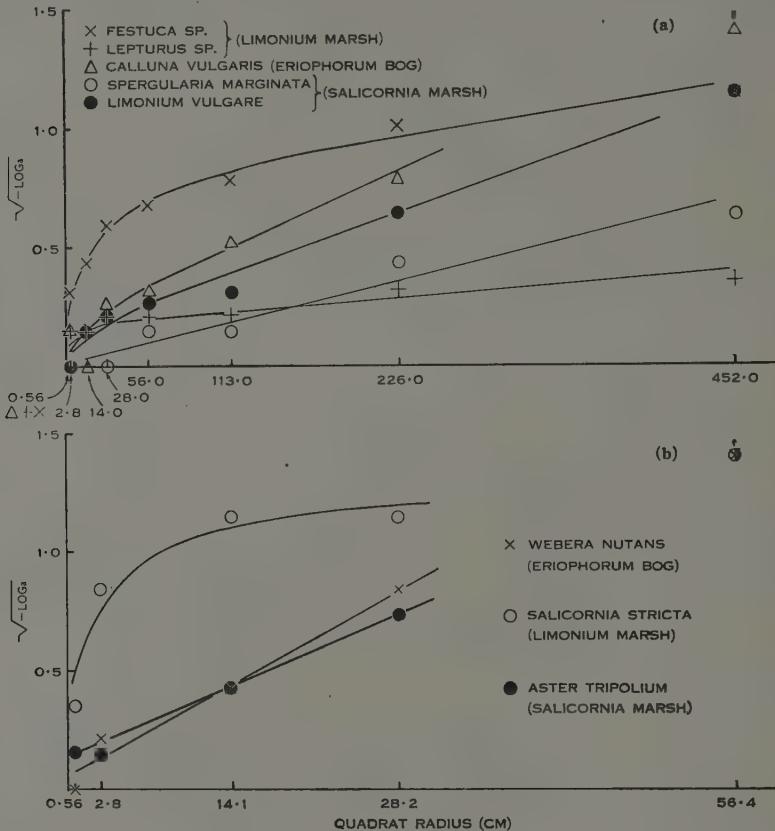


Fig. 4.—Graphs demonstrating the relationship between $\sqrt{-\log a}$ and quadrat radius for a number of species; from Archibald (1952). The sign ↑ attached to a symbol indicates that 100 per cent. presence has been recorded for the species for that particular quadrat. For purposes of graphing a 99 per cent. presence has been assumed.

an aggregation circle is $\exp(-\pi r_2^2 d_2)$. However, not all of the area within an aggregation circle is covered. Within any one such circle, the probability of a point quadrat not contacting a unit is $\exp(-\pi r_1^2 d_1)$. Let p_1 and q_1 be respectively the proportions of an aggregation circle that are occupied, and not occupied, by the units. Similarly let p_2 , q_2 be the proportions of the entire area occupied, and not occupied, by the aggregation circles. Then the proportion of the entire area occupied by the units is $p_1 \times p_2$. The probability that a point quadrat will not contact a unit is the

$$*\exp(-\pi r_2^2 d_2) = e^{-\pi r_2^2 d_2}.$$

proportion (of the entire area) which is not occupied by units, viz. $1 - p_1 p_2$. Let a (absence) be this total probability. Then

$$\begin{aligned} a &= 1 - p_1 p_2 \\ &= 1 - (1 - q_1)(1 - q_2) \\ &= q_1 + q_2 - q_1 q_2 \\ &= q_2 + q_1(1 - q_2) \\ &= q_2 + q_1 p_2 \\ &= \exp(-\pi r_2^2 d_2) + \exp(-\pi r_1^2 d_1) [1 - \exp(-\pi r_2^2 d_2)]. \end{aligned}$$

If a circular quadrat of radius R is used, then any portion of a plant unit at a distance $\leq R$ from any position of the original point quadrat is now contacted and recorded as present. The same result is obtained if a point quadrat is retained, and the boundaries of each plant unit advanced a distance R towards the point quadrat, i.e. the plant unit radius is increased by R . The effective radius of the aggregation circle will also be increased by R , as its outer limits are fixed by the outer limits of the two or three outlying plant units. The total probability of no contacts, i.e. absence, is now expressed by the equation

$$a_c = \exp[-\pi(R+r_2)^2 d_2] + \exp[-\pi(R+r_1)^2 d_1] [1 - \exp\{-\pi(R+r_2)^2 d_2\}], \quad \dots (6)$$

where a_c is the probability of absence if the plant units are aggregated.

The relationship between values of a_c and a (calculated from equation (1) for random distribution) is demonstrated from an actual example. The following values are used: $r_1 = 1.0$, $d_1 = 0.1$, $r_2 = 20.0$, $d_2 = 0.001$. The overall density of the plant units is therefore $d_1 d_2$. The calculated values of a_c and a are now plotted against the corresponding values of the quadrat radius R . Figure 5 shows the curve Ia resulting from equation (6), and the straight line I from equation (1). The significance of the curves shown in Figures 4(a) and 4(b) is now clear. The departure from the straight line is indicative of the aggregation of the plant units. Aggregation always results in the proportion of quadrats with no individuals being greater than expected, with the value of $\sqrt{-\log a}$ being consequently lower than for the random distribution.

The assumptions which were made in deriving equation (6) are not exactly true for all contagious distributions. Within each aggregation circle the units have been assumed to be distributed at random, whereas in fact the density is more likely to be greatest towards the centre and least at the circumference. In this latter case the curve obtained from plotting $\sqrt{-\log a}$ against the corresponding quadrat radius would follow a path slightly below Ia in Figure 5. This difference does not invalidate equation (6), but the limits of the underlying assumption should be kept in mind.

The relationship of curve Ia to the various constants is also shown in Figure 5. Line I is the result of plotting $\sqrt{-\log a}$ against quadrat radius R for a random distribution of plant units of radius r , and density $d_1 d_2$. Line II is the result of plotting $\sqrt{-\log a}$ against quadrat radius R for a random distribution of units equal in size and density to the aggregation circles. In this latter instance a is the probability of a quadrat neither falling within nor touching any aggregation circle. Curve Ia, while having a common point, $(-r_1, 0)$, with line I, departs progressively from that

line to become practically identical with line II for high values of the quadrat radius. The rate of departure is apparently some function of the degree of aggregation and the quadrat radius R . The important part of the curve is the early section, particularly the intersections with the x - and y - axes, from which the unit size and the area covered can be calculated.

Two equations similar to equation (1) were derived earlier by the author as an aid in the study of the distribution of fungal material in soils and on plant roots. They deal with sampling in three dimensions (Aberdeen 1955) and along a cylindrical surface (Aberdeen 1956). Both these equations can now be adjusted to allow for contagious distribution when they take the same general form as equation (6).

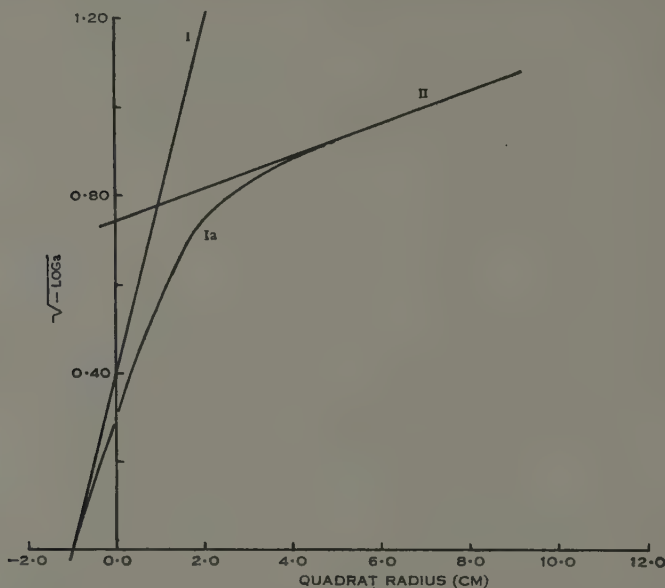


Fig. 5.—Graph showing relationships of the curve from equation (6). Line I shows relationships for the random distribution of plant units. Line II demonstrates the relationships for the random distribution of units equal in size to the circles of aggregation.

III. DISCUSSION

The criticisms of frequency estimates arise mainly from a feeling of uncertainty. The method has been justified empirically in certain circumstances (e.g. see Blackman 1935), but there has been no clear demonstration of its limitations. Using the relationships discussed in the previous section, a much clearer conception is now possible.

In practice, the total area covered by each species, and the density of its units, are probably the most important quantitative characteristics of a plant community. The first can be estimated directly by adequate sampling with point quadrats, and the result obtained is independent of random or contagious distribution. The peculiar difficulties associated with such techniques are discussed by Goodall (1952*b*) and

Robinson (1955), among others. However, the method gives no estimate of density, and it is only practicable when total coverage is very high.

With one exception, frequency values derived from a single size of quadrat of significant dimensions give no information on the details of the pattern of a particular species. The exception is the random distribution of plant units of negligible size, when the density can be calculated directly from the frequency. On the other hand, consideration of the curves shown in Figures 4(a) and 4(b) above, shows that a significant difference between the frequency values of two species for a single size of quadrat can be used to demonstrate that there is *some* difference in their overall patterns. However, it does not indicate in which of the species characteristics, viz. size, distribution, density, or area covered, the difference is to be found. On the other hand, equal frequencies do not necessarily mean that any characteristics of the two species are equal. If the distribution of a species is assumed to be random and an estimate of the plant unit size is also made, then the frequency value from a single quadrat can be used to give an indication of the total area covered, the density, and the size of the plant units.

Two sizes of quadrats, if the distribution is known to be random, would supply the necessary data for calculating all the population characteristics. If the distribution is unknown, and a random arrangement is assumed, then a reasonable approximation of area covered could be obtained from two quadrat sizes, if the areas of the quadrats are small. Further extrapolation beyond the y -axis would not be justified.

However, if we concentrate on differences between species, then the results from two quadrat sizes can be used to differentiate patterns in a more precise manner. A statistically significant difference for one or both quadrats means a significant difference between the patterns being compared. If the absence values for two sizes of quadrat are combined with an estimate of plant unit size, then some indication of random or contagious distribution is possible. In any case it will increase the usefulness of the other estimates, even if a random distribution is assumed.

If frequencies alone are to be used to differentiate random and non-random distributions, then the theoretical minimum number of quadrat sizes is three to detect departure from linearity on a graph, and four for equation (6). Either of these possibilities requires that the corresponding values of a should be determined very accurately, which would necessitate a very large number of replications for each quadrat size. A more workable method would be to increase the number of quadrat sizes used, and reduce the replications. It is not possible, however, to make any definite recommendations. The final decision depends on the conditions of the experiment and the amount of information required. The results from Archibald's experiments illustrate the difficulties. For her model population she used 60 replications for each of seven quadrat sizes and she was working under ideal conditions. A reconsideration of Figure 3 and Table 1 demonstrates the variation that is likely to occur even in those circumstances. The values for density, calculated from the slopes of the regression lines, showed the least departure from expectation. On the other hand, the field results shown in Figures 4(a) and 4(b) demonstrate that departures from random distribution can be detected in practice if a suitable number of quadrat sizes are used. Twenty replications of eight quadrat sizes were used in these latter trials.

The main difficulty associated with the non-random distribution is the need to extrapolate to obtain the x and y intercepts. This is largely overcome if an estimate of plant unit size is made at the same time as the frequencies are being taken. With the x intercept so fixed, the crucial y intercept (for determination of total area covered) can be obtained by interpolation. The density is readily estimated from the slope of the line for a random distribution but cannot be easily estimated for the contagious distribution. An approximation could now be obtained, however, by using the slope of the straight line joining the x and y intercepts.

IV. ACKNOWLEDGMENT

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V. REFERENCES

- ABERDEEN, J. E. C. (1954).—The estimation of basal or cover areas in plant ecology. *Aust. J. Sci.* **17**: 35–6.
- ABERDEEN, J. E. C. (1955).—Quantitative methods for estimating the distribution of soil fungi. *Univ. of Qd. Pap. (Bot.)* **3**: 83–96.
- ABERDEEN, J. E. C. (1956).—Factors influencing the distribution of fungi on plant roots. I. Different host species and fungal interactions. *Univ. of Qd. Pap. (Bot.)* **3**: 113–24.
- ARCHIBALD, E. E. A. (1948).—Plant populations. I. A new application of Neyman's contagious distribution. *Ann. Bot. Lond. (new ser.)* **12**: 221–35.
- ARCHIBALD, E. E. A. (1952).—A possible method for estimating the area covered by the basal parts of plants. *S. Afr. J. Sci.* **48**: 286–92.
- ASHBY, E. (1935).—The quantitative analysis of vegetation. *Ann. Bot. Lond.* **49**: 779–802.
- BLACKMAN, G. E. (1935).—A study by statistical methods of the distribution of species in grassland associations. *Ann. Bot. Lond.* **49**: 749–77.
- BROWN, DOROTHY (1954).—Methods of surveying and measuring vegetation. Commonwealth Bureau of Pastures and Field Crops, Bull. No. 42.
- GOODALL, D. W. (1952a).—Quantitative aspects of plant distribution. *Biol. Rev.* **27**: 194–245.
- GOODALL, D. W. (1952b).—Some considerations in the use of point quadrats for the analysis of vegetation. *Aust. J. Sci. Res. B* **5**: 1–41.
- HOELL, P. G. (1943).—The accuracy of sampling methods in ecology. *Ann. Math. Statist.* **14**: 289–300.
- PRESTON, F. W. (1948).—The commonness, and rarity, of species. *Ecology* **29**: 254–83.
- ROBINSON, P. (1955).—The estimation of ground cover by the point quadrat method. *Ann. Bot. Lond. (new ser.)* **19**: 59–66.
- WILLIAMS C. B. (1950).—The application of the logarithmic series to the frequency of occurrence of plant species in quadrats. *J. Ecol.* **38**: 107–38.

DARK ISLAND HEATH (NINETY-MILE PLAIN, SOUTH AUSTRALIA)

VI. PYRIC SUCCESSION: CHANGES IN COMPOSITION, COVERAGE, DRY WEIGHT, AND MINERAL NUTRIENT STATUS

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Summary

The dynamic changes in the composition, dry weight, and mineral nutrient status of heath following fire have been investigated.

The overall growth (dry weight/time) curve for the aerial organs of the heath is essentially exponential.

Soil moisture is conserved by burning and, provided climatic conditions are favourable, regeneration of all species is rapid. Annual species are rare and are found only in the first year after a fire. Many species are fire-resistant and regenerate rapidly from buried perennating buds; the others reproduce in great numbers from seeds. The number of propagules varies with the age and composition of the parent stand.

The initial regrowth, dominated by *Xanthorrhoea australis*, produces annually over 500 kg dry weight per acre.

Two or three years after a fire the regrowth of *Casuarina pusilla* and a wealth of undershrubs form a large part of the stand. Growth is much slower with only 240 kg dry weight produced annually per acre. During this period many species or the understorey reach their peak and die; the major species are reduced in number. This decrease in numbers may be through natural senescence of the species, but is certainly hastened by competition for water and, to a lesser extent, light. It continues throughout the development of the heath.

After about 10 years, the numerous seedlings of *Banksia ornata* dominate the stand, probably owing to reduced competition from the understorey plants. A dry weight of 180 kg per acre is produced annually over the next 5 years. After this time (15 years) there is a continuous fall in the annual growth rate to 160 kg per acre towards the 50-year period. Of 36 species recorded after a fire only 20 persisted after 25 years, five of these 20 contributing less than 1 kg dry weight per acre. Only ten of these species persist after 50 years and most of these are greatly depleted in numbers. Almost 15,000 kg dry weight per acre were found in the 50-year stand dominated by massive plants of *B. ornata* and *X. australis*.

Apart from the first 10 years when the underground organs contribute considerable food reserves to the regenerating aerial organs, the evidence suggests that these organs increase in dry weight per acre almost as much as those above ground.

Nutrients from this very infertile soil, the Makin sand, steadily accumulate in the underground organs, often at the expense of the aerial organs. Translocation of many nutrients (P, N, K, Ca, Cu, Zn, and Mn) to aerial organs may be greatly reduced, that of some elements almost to zero. This must contribute greatly to the decreasing growth rate of the aerial organs. As *Casuarina* and *Phyllota* spp. contain greater concentrations of nutrients than the other species their requirements are presumably greater. They are eliminated early under nutritional stress. Gradually only those species survive in which the concentration of nutrient elements is low, namely *Banksia* spp. and *Xanthorrhoea*. However, as over 50 per cent. of the nutrients in the aerial organs of these species are bound in fruits and dead leaves, even these species must

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suffer nutrient stress and degradation of the stand must inevitably occur, to be followed by regeneration on the release of the nutrients.

The frequency of fire is such that the heath does not mature. Regular firing is essential to maintain many elements of the flora.

Changes in the nutrient levels of soil and litter are also indicated.

I. INTRODUCTION

In previous papers (Rayson 1957; Specht 1957*a*, 1957*b*; Specht and Rayson 1957*a*, 1957*b*) the heath vegetation and its environment at Dark Island in the upper south-east of South Australia have been discussed. In the present paper, attention is centred on regeneration of vegetation after a fire and on dynamic changes in composition, dry weight, and mineral nutrient status of the heath.

II. FREQUENCY OF FIRES

Coaldrake (1951) recorded the long history and frequent occurrence of fires in the area; even before the advent of white settlers fires were started by aboriginal hunters and by lightning. When heath is 4-5 years old, it has sufficient standing vegetation and litter to carry a fire and if the fire has gathered impetus, younger stands may be burnt. Wind change, calms in the shelter of sand dunes, tracks and fire-breaks, and local high vegetation densities alter the extent and direction of fires. This results in uneven fronts and a great complexity of fire patterns.

Times of high fire risk do not necessarily correspond with times of climatic stress on the vegetation. Several hot, windy days are sufficient to dry the surface and to make the fire risk high despite high soil moisture reserves.

III. THE IMMEDIATE EFFECT OF FIRE

Fire razes all the aerial growth of the heath except the main branches of some of the larger, woody species. Species which have perennating buds above the surface of the ground are killed outright, but species with some perennating buds below the soil surface regenerate from these (Specht and Rayson 1957*b*).

It is only after fire that the follicles of *Banksia ornata** open and release their seeds; fire, as well as slow desiccation during summer, also releases the seeds from the woody fruits of *Casuarina* and *Leptospermum*. Heat may be beneficial to the maturation of seeds in these as in other sclerophyllous species (Sampson 1944). *Helichrysum* spp. and *Stipa McAlpinei* germinate only after a fire.

Leaf litter is entirely burnt. Lichens and soil algae, which normally form a crust over the otherwise bare soil between bushes, are scorched and tend to break up leaving the soil bare to erosion. Wind erosion may be considerable, particularly in exposed sites. Mounds of sand accumulate at the junction of burnt and unburnt stands of heath, entire sand dunes drift slightly, and ash is redistributed. Rainstorms with high falls over a short period cause gullying on dune faces. Both wind and water cause the depletion of seeds and ash from exposed hillsides and hillocks and the accumulation of both in hollows between old stocks or around litter of unburnt sticks. Light rains following a fire minimize erosion and rehabilitate the soil crust organisms.

*Nomenclature follows that used in Part I of this series (Specht and Rayson 1957*a*, Appendix I).

IV. INITIAL STAGE IN HEATH REGENERATION

(a) *Composition of the Parent Stand*

It was found that local aggregation of parent species affected the distribution of propagules whether they were seeds or underground stocks. Natural aggregation occurred when the plant possessed vegetative means of reproduction. Microtopography had a marked effect on the local densities of the various species (Rayson 1957).

The age of the stand at the time of burning affects the number of propagules available. Fires at intervals of less than 5 years are found to eliminate or reduce numbers of *Banksia*, *Casuarina*, and *Leptospermum*, which take several years to reach reproductive maturity (compare Plate 2, Figs. 1 and 2).

Marked differences were found in the species composition of the seedlings which germinated after two adjacent stands of heath were burnt. One of those stands was 6 years old, and the other 10 years since a previous fire. In the 6-year stand, 15,400 seedlings of early-maturing species and 45,400 seedlings of late-maturing species germinated per acre; the 10-year stand showed fewer early-maturing species, namely 5100, and many more late-maturing species, namely 153,100 per acre. If no fire occurs for at least 20 years, many species may have been eliminated from the stand during the pyric succession (see Section V(c)). Some seeds of these species may have accumulated in the soil and still be viable but the chance of survival decreases with the age of the stand.

(b) *Germination Optima*

(i) *Temperature*.—Triplicate sets of 25 seeds of *B. ornata* were germinated over a 6-week period at temperatures of 5, 10, 15, 20, 25, and 30°C. This species, which regenerates solely from seeds, showed 0, 0, 75, 72, 69, and 0 per cent. germination respectively at the different temperatures. The radicles of half the viable seeds emerged in 20 to 23 days from the beginning of the test. The cotyledons took twice as long to emerge.

A similar test with *Casuarina pusilla*, which regenerates from both rootstocks and seeds, showed 0, 11, 9, 9, 8, and 0 per cent. germination respectively at the above temperatures. Half the viable seeds germinated in 23 days at 10°C, in 13 days at 15°C, in 10 days at 20°C, and in 7 days at 25°C. Maximum germination occurred 1 or 2 days later.

Visual observations indicated that, after germination, maximum growth occurred at 15°C for both species.

Records of the maximum and minimum air and soil temperatures for Keith (Specht and Rayson 1957a) show that temperatures may be suitable for germination from late April to early June and from mid September to early October each year. Minimum terrestrial temperatures, however, are lower in September (2.8°C) than in April or May (3.3°C and 4.4°C respectively) and the frost risk is higher.

(ii) *Moisture*.—The summer deficit of water was estimated to occur for an average of 2–3 months of the year from February to April (Specht 1957b). However, even when there was an excess of soil moisture in the profile throughout the year, the

moisture status of the surface was critical for the germination of seeds. Optimal conditions for germination depend on:

- (1) A fall or falls of rain sufficient to constitute a definite break in the season.—Field observations indicated that light showers of rain were rapidly utilized by the established perennial vegetation and were therefore inadequate for the germination of seeds. A definite break in the season has been somewhat arbitrarily defined as falls of rain sufficient to store 1 in. of water in the soil during the first month after the drought.

The number of times the influential winter break (defined as above) occurred in any particular month during the last 48 years is as follows:

| | | | |
|--------|----|----|----|
| March | .. | .. | 2 |
| April | .. | .. | 6 |
| May | .. | .. | 27 |
| June | .. | .. | 10 |
| July | .. | .. | 2 |
| August | .. | .. | 1 |

- (2) Rain falling within the months of optimal temperature for germination (April and May).—The germination rate of seeds of the heath species was slow in comparison with that of crop and pasture plants. Optimal germination therefore occurred when moisture was adequate throughout the whole of the period of suitable temperature. The onset of low winter temperatures, shortly after germination occurred, killed the seedlings before they appeared above the ground. Hard seeds failed to germinate until September or the following year. The distribution of the dates of the effective winter break showed that once in 16 years the lateness of the break made any germination unlikely, while in approximately one in four years optimal temperature and moisture did not coincide.

(c) *Survival of Seedlings*

Moisture and temperature are the two factors principally concerned in the survival of seedlings during the first seasons as well as in germination.

(i) *Winter Drought*.—It is possible, in the event of subnormal following rains, for surface conditions to be dry while deep-rooting plants are supplied with adequate water. Seedling counts made on recent burns in 1950, 1951, and 1954 showed 61,100, 504,800, and 497,300 seedlings per acre respectively. In 1950, many seedlings died owing to irregular, inadequate following rains.

(ii) *Summer Drought*.—Yearly seedling counts on the permanent quadrats established on two burns showed that the loss of *B. ornata* seedlings over the first summer was 60 per cent. in 1950 and 42 per cent. in 1951. With or without favourable moisture conditions, these seedlings have a high mortality rate during the first year. In their first season 80 per cent. of all seedlings other than *B. ornata* were lost in 1950 and 12 per cent. in 1951. Loss during the next 5 years was nil in *B. ornata* and less than 15 per cent. in all other species.

(iii) *Frost*.—Following a severe frost in October 1954, approximately 34 per cent. of *B. ornata* seedlings in the first year of development died. Moisture was adequate at the time.

V. LATER TRENDS IN HEATH REGENERATION

The life-span of some of the perennial heath species is too long for studies on a single permanent area to be carried out at present. The stands of various ages described in this paper were distributed within an area of half a square mile; climate and soil were identical. All stands were at a sufficient distance from larger dunes to minimize the effect of microclimate. The only variables impossible to control between stand and stand were the previous history of burning and the initial conditions for regeneration.

(a) *Methods*

(i) *Age of the Stand*.—Where the date of the preceding fire was unknown the age of the stand was determined by calculating the maximum number of annual growth increments of *Banksia marginata*. An even-aged population of aerial shoots regenerates after a fire and relatively few subsidiary sucker plants develop during the first 20 years of heath regeneration. Between 20 and 30 years, until their final degeneration, parent plants are easily recognizable from the suckers.

(ii) *Percentage Cover of Individual Species and the Extent of Bare Ground*.—These were estimated from 25 random line transects, each 22 yd in length, on stands of 2½, 5, 6, 9, 12, and 25 years since the last fire. Transects were recorded according to the line interception method used by Canfield (1941, 1944), Parker and Savage (1944), and Roe (1947).

(iii) *Numbers of Plants*.—Within stands of 2½, 9, 15, and 25 years, all the large phanerophytes, viz. *Banksia ornata*, *B. marginata*, *Xanthorrhoea australis*, *Casuarina pusilla*, *Leptospermum myrsinoides*, *Phyllota pleurandroides*, *Spyridium subochreatum*, and *Adenanthos terminalis* were counted on six random 5 by 10 yd quadrats. Each 5 by 10 yd quadrat was subdivided into a 2 by 5 yd area on which seedlings of the above species and all other species were counted. Seedlings and regrowth plants were separated in all cases. Mat sedges and grasses were not counted because of the impossibility of deciding the extent of a single plant. The size and number of the quadrats was chosen to give a representative sample of all species (Specht and Rayson 1957a).

(iv) *Dry Weight of Aerial Parts of Vegetation*.—Tops of all plants were harvested from the above quadrats, separated into species and weighed after drying to constant weight at 95°C. Subdivision of all species into regrowth and seedlings, of *Xanthorrhoea* into dead and living leaves, and of *B. ornata* and *B. marginata* into fruit and leaf-stem fractions was made. All the litter was collected from each 2 by 5 yd quadrat, dried, and weighed.

(v) *Average Height*.—The average heights of the major species were calculated from the measurements of 20 randomly chosen plants within each stand.

(vi) *Supplementary Data*.—A small stand of heath, at least 50 years of age according to the local residents, was discovered 10 miles due north of Meningie at the

edge of the Ninety-Mile Plain. It occurred on an isolated pocket of deep sand within a *Casuarina stricta* savannah woodland formation. Though the species composition was very variable one portion of it contained mostly those species which were found on the sand plain at Dark Island. A quadrat of 5 by 10 yd, the size used in the study

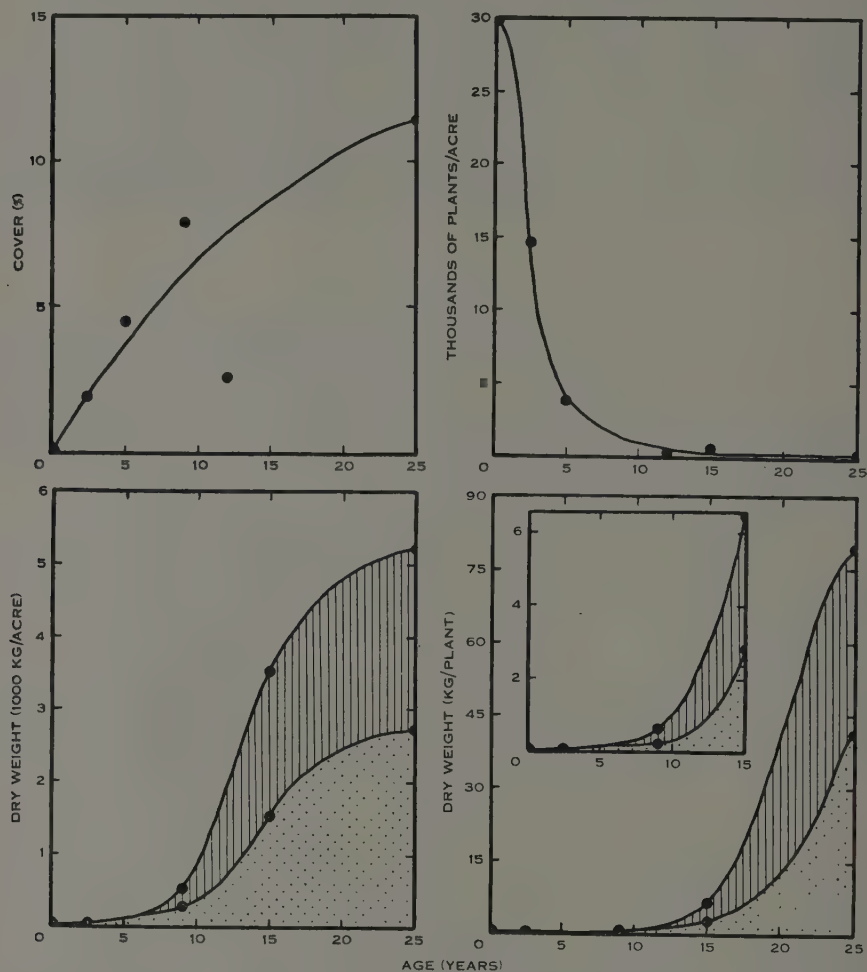


Fig. 1.—Development of *Banksia ornata* in cover, numbers, and dry weight over a 25-year period after burning. Shaded areas denote fruits; stippled areas, leaf-stem fraction. The 50-year stand showed 190 plants per acre contributing 12,920 kg dry weight of which only 3960 kg were leaves and stems.

at Dark Island, was inadequate in sampling this stand. Some of these quadrats contained only very large clumps of *Xanthorrhoea*, others a group of *B. ornata* specimens, others mainly bare ground. Nevertheless, the mean values of the numbers of plants per acre, the dry weight per plant, and the dry weight per acre were obtained from several of these quadrats to give some indication of the development of the heath vegetation after 25 years of age. It must be stressed that this stand was not completely

comparable with those at Dark Island. The Meningie sand dunes supported more *B. ornata* than the sand plains and were completely devoid of *B. marginata* (Rayson 1957). Also, the annual rainfall at Meningie is approximately 2 in. lower than that at Dark Island.

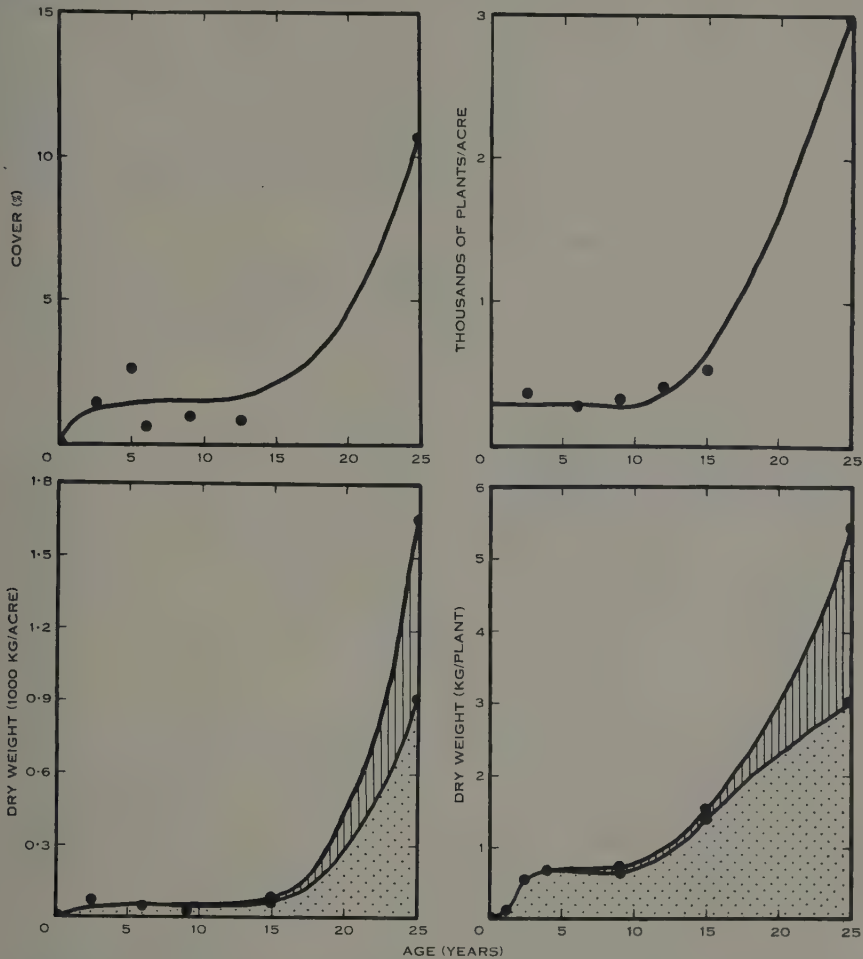


Fig. 2.—Development of *Banksia marginata* in cover, numbers, and dry weight over a 25-year period after burning. Shaded areas denote fruits; stippled areas, leaf-stem fraction.

(b) Results

Species of major importance are dealt with separately in order of their relative importance in the 25-year stand. Less important species are grouped according to their life histories. With the exception of *Spyridium*, which reached a height of 2 ft, plants referred to as “understorey” did not exceed 12 in. height at 25 years.

(i) *Banksia ornata* (Fig. 1).—The number of seedlings decreased very rapidly during the first 5 years of growth and was reduced to approximately one-quarter in each successive 5-year period. Cover and dry weight increased to 11 per cent. and

48 per cent. respectively in the 25-year stand. The discrepancy between these figures was due to a decrease in the leaf-stem ratio and the accumulation of woody fruits. The average height of 25-year old bushes was 8 ft.

Plate 4, Fig. 1 shows a degenerating *B. ornata* bush, one of a number found in fragments of heath between 40 and 50 years of age. As healthy bushes of this age have also been found, the complete degeneration of an even-aged stand of *B. ornata* may take many years. Until this age there was no evidence that seeds were released at any time except after a fire. Following the degeneration of the bushes, the fruits shed may dry out and open. Only one or two such bushes have been found surrounded by a few seedlings. Further observations are needed to establish the fact that a continuous process of growth, death, and effective regeneration of *B. ornata* would occur after very long periods in undisturbed heath.

(ii) *Banksia marginata* (Fig. 2).—Rapid regeneration of vegetative parts occurred from underground stocks. Although flowers were initiated each year, the number of flowers fertilized in each inflorescence was very low; few seeds were shed and seedlings were exceptional.

The number of plants remained constant during the first 15 years of growth and contribution to both cover and total dry weight was only 1 per cent. Subsequent development was rapid. The height of bushes was doubled and reached an average of 5 ft in the 25-year stand. Numbers of sucker plants developed from lateral roots of the adults and the cover increased to 11 per cent. In contrast to *B. ornata*, fruits of *B. marginata* contributed little to dry weight until the plants were approximately 20 years old. When fruiting maturity was reached the dry weight increased to 15 per cent. From 25 years onward parent plants tended to die out, leaving the surrounding thickets of vigorous suckers (Plate 4, Fig. 2).

(iii) *Xanthorrhoea australis* (Fig. 3).—Regeneration of this species from underground stocks followed almost immediately after a fire (Coaldrake 1951; Specht and Rayson 1957a). Plate 1, Figure 1 shows a stand of heath 1 month after a fire, with *Xanthorrhoea* plants already 12–18 in. high. This species contributed 90 per cent. of the aerial growth during the first year. Flowering occurs only after a fire and is restricted to plants growing on sand ridges (Plate 1, Fig. 2). The seeds are attacked by insect larvae and no seedlings have been found.

Additional counts were made of the number of plants per 50 sq. yd in the stands of four ages and an analysis of variance carried out with a total of 37 degrees of freedom. There was no significant difference in numbers of plants with age ($P > 20$ per cent.). Vegetative division of stocks must have approximately balanced the death of plants.

Dead leaves remain attached to the plants for an indefinite time, becoming semi-prostrate and increasing the percentage cover considerably (Plate 3, Fig. 2).

(iv) *Casuarina pusilla* (Fig. 4).—The importance of seedlings in the regeneration of this species is difficult to assess as the numbers present in the stands harvested varied considerably. Numbers in the 2½- and 9-year stands gave a 7:3 ratio, but the previous history of the two stands was unknown. No recognizable seedling plants or recent seedlings were found in older stands.

Regeneration from rootstocks was rapid; within 3 years the bushes covered almost as much area as before the fire. Though the maximum coverage occurred between 5 and 7 years, it remained more than 20 per cent., the highest for any species,

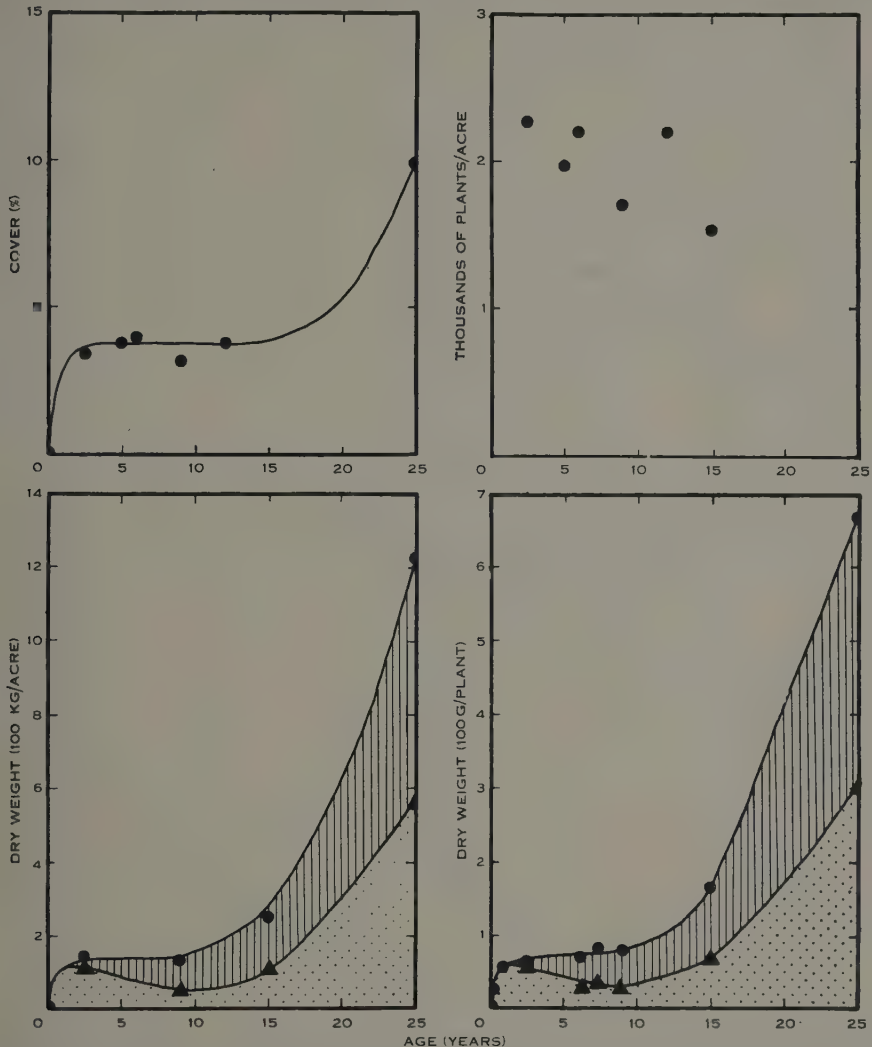


Fig. 3.—Development of *Xanthorrhoea australis* in cover, numbers, and dry weight over a 25-year period after burning. Stippled areas denote living leaves; shaded areas, dead leaves. The 50-year stand showed 1740 plants per acre contributing 1800 kg dry weight of which only 570 kg were living leaves.

from 2 to 15 years; from 3 years onward, growth was more in height, secondary thickening, and fruits than in diameter. Average height was 2 ft at 15 years and 4 ft at 25 years.

The number of regrowth plants per acre decreased linearly with age ($b = -393.7$, $P < 0.1$ per cent.). Although the dry weight per plant continued to increase

the death rate was rapid enough to decrease both cover and total dry weight in the 25-year stand. If this rate of decrease were maintained, the species would die out entirely between 30 and 35 years after a fire, though a rare specimen was found in the 50-year stand.

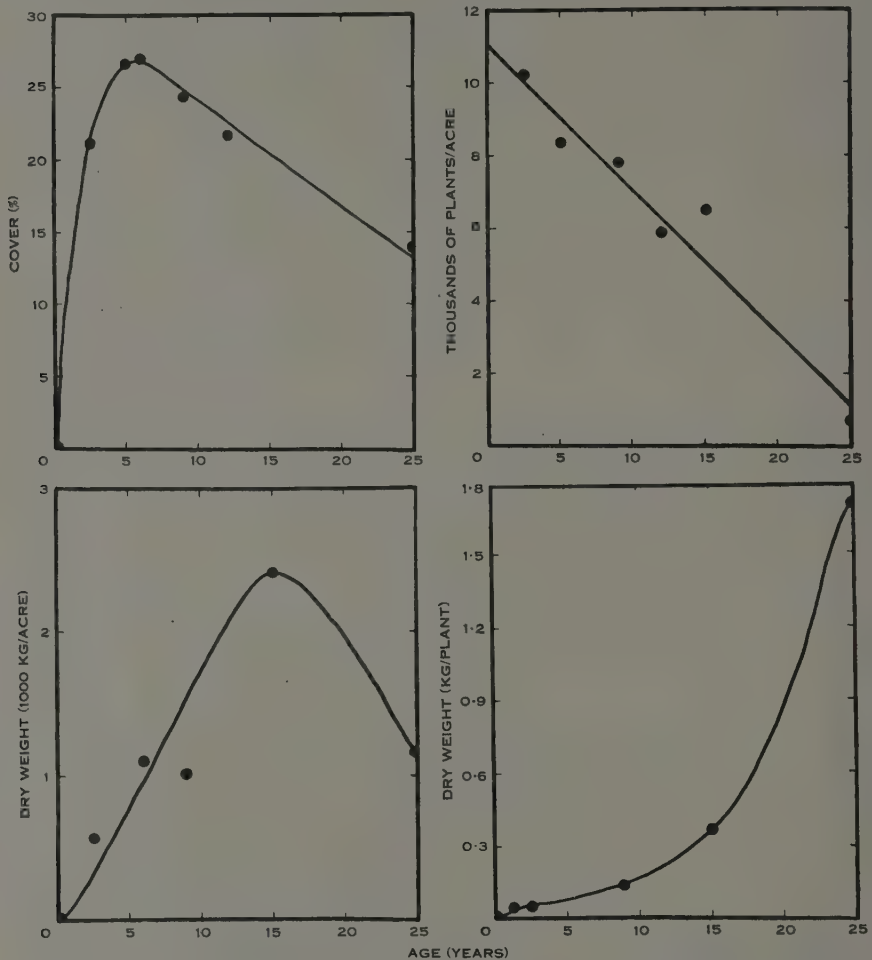


Fig. 4.—Development of *Casuarina pusilla* in cover, numbers, and dry weight over a 25-year period after burning. *Casuarina* was rare in the 50-year stand. The one specimen harvested weighed 5.2 kg when oven-dried.

(v) *Leptospermum myrsinoides* (Fig. 5).—Seed and regrowth regeneration were equally important; 90 per cent. of the seedlings present in the 2½-year stand were established within 9 years and indistinguishable from regrowth plants. This explained the low dry weight per 9-year plant.

From 10 years onward the number of adult plants decreased. Fruits and leaves were shed from the dead plants but the woody skeletons remained, forming much of the litter in the 25-year stand. The growth of the remaining plants was rapid enough

to increase the dry weight from 1 per cent. to 10 per cent. between 10 and 25 years. Cover was higher than for any other species in the 25-year stand. As the undergrowth died out, *Leptospermum* seeds germinated. There was no evidence of seedling regeneration in the 50-year old stand; if they had germinated as in the 25-year stand they had not survived.

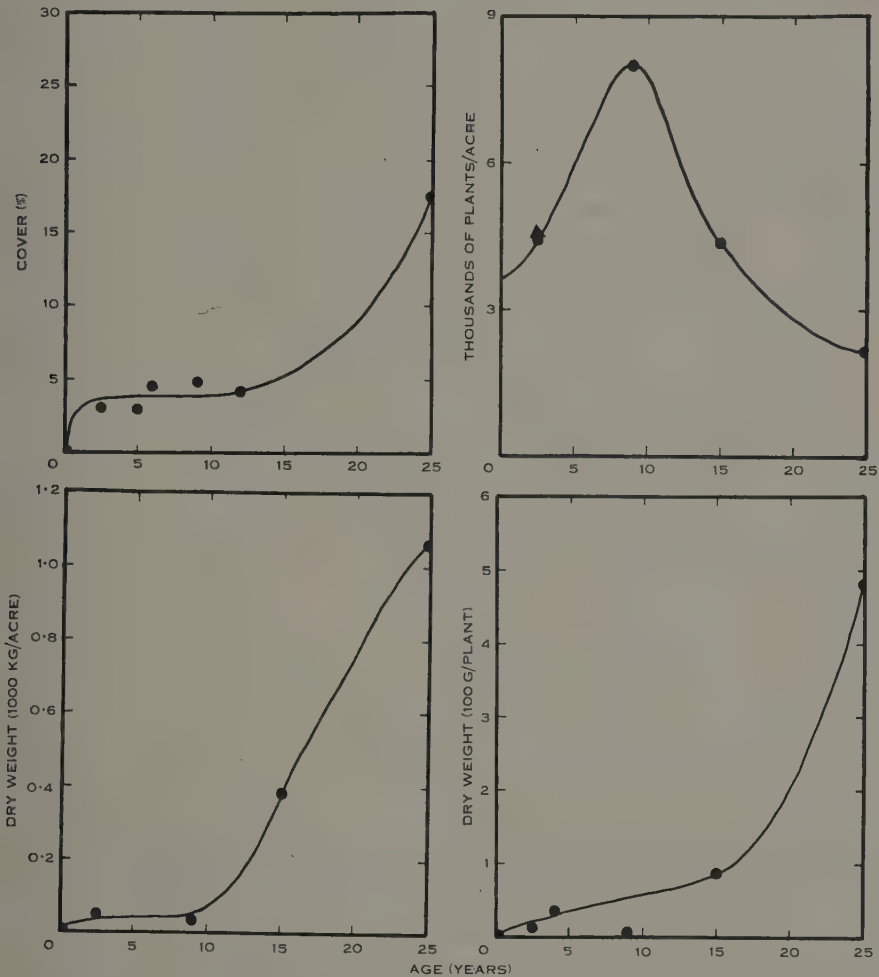


Fig. 5.—Development of *Leptospermum myrsinoides* in cover, numbers, and dry weight over a 25-year period after burning. Triangles in number-time curve indicate number of seedlings.

(vi) *Phyllota* spp. (Fig. 6).—*Phyllota remota* regenerates solely from seeds. It was an important component of the heath in some localities for the first 15 years of growth. Thereafter it died out completely; it was not found in 25-year heath in areas favourable to its development and surrounded by younger stands containing it.

Phyllota pleurandroides rarely exceeded 1 ft in height and contributed little to cover or dry weight for 15 years, though aerial shoots continued to increase in number.

Isolated bushes 4 ft in height and of tremendous size compared with 15-year shoots have been found in mixed heath of uncertain age. This form could therefore attain importance in aging stands which are topographically favourable to it.

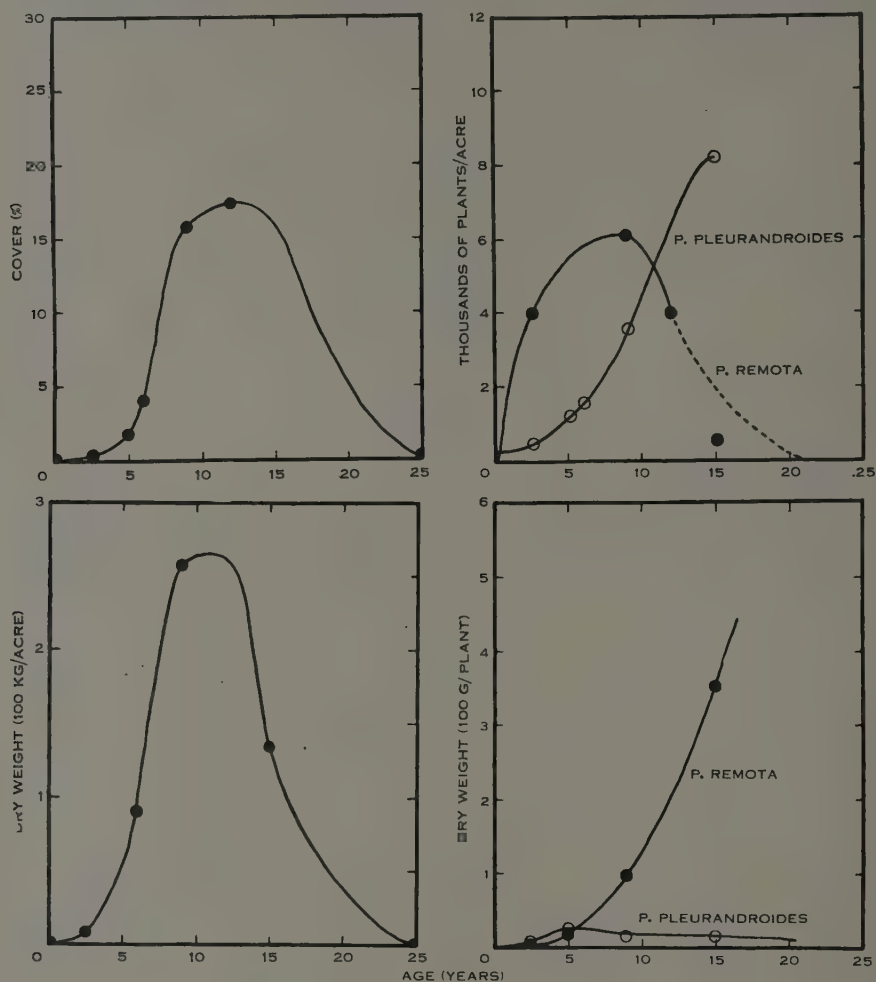


Fig. 6.—Development of *Phyllota* spp. in cover, numbers, and dry weight over a 25-year period after burning.

(vii) *Group A: Understorey Plants of Maximum Importance Directly after a Fire* (Fig. 7).—These bind the sand between larger bushes while the undershrubs become established. The group may be further subdivided into:

Herbs which die out within 3–4 years, viz. *Helichrysum obtusifolium*, *Helichrysum blandowskianum*, *Stipa McAlpinei*, and *Poranthera microphylla*. *H. obtusifolium* accounted for 65 per cent. of the whole. *Stipa* and *Poranthera*, both annuals, are found only in the first year after a fire if moisture is adequate.

Plants which retain some importance for 15–20 years and persist in the 25-year stand, viz. *Lepidosperma laterale*, *Boronia caerulea*, *Schoenus tepperi*, *Amphipogon caricinus*, *Danthonia setacea*, *Neurachne alopecuroides*, and *Stipa semibarbata*.

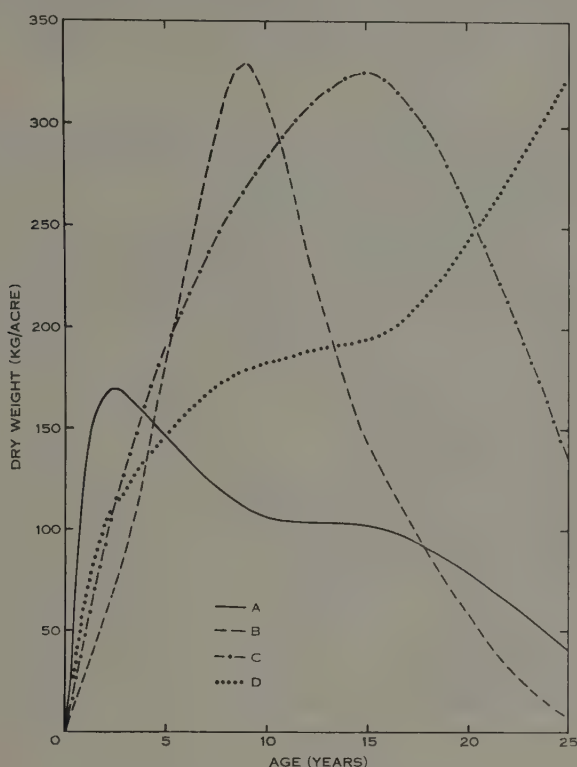


Fig. 7.—The total dry weight in kg of the understorey plants of Groups A, B, C, and D over a 25-year period after burning.

(viii) *Group B: Undershrubs of Maximum Importance Approximately 10 Years after a Fire* (Fig. 7).—Again the group may be subdivided into:

Species which regenerate from seeds, viz. *Leucopogon costatus*, *Leucopogon woodsii*, *Baeckea ericaea*, *Cryptandra tomentosa*, *Gompholobium minus*, and *Adenanthos terminalis*. These were insignificant in 15-year, and absent from 25-year, understorey.

Species which regenerate from seeds and rootstocks, viz. *Spyridium subochreatum*, *Daviesia brevifolia*, *Lomandra juncea*, and *Dillwynia hispida*. These attained importance earlier and persisted longer than the preceding division.

(ix) *Group C: Understorey Plants of Maximum Importance Approximately 15 Years after a Fire* (Fig. 7).—This group includes *Hibbertia stricta*, *Hibbertia sericea*, *Astroloma conostephioides*, and *Lepidobolus drapetocoleus*.

A total of 5500 recent seedlings per acre were recorded in the 25-year stand; a second period of importance could occur as heath ages. *Hibbertia stricta* was numerically the commonest undershrub at any time.

(x) *Group D: Understorey Plants Still Increasing in Importance 25 Years after a Fire* (Fig. 7).—This group includes: *Hypolaena fastigiata*, *Lepidosperma carphoides*, and *Calytrix alpestris*. Initial regeneration was rapid but development of these was delayed between 5 and 15 years.

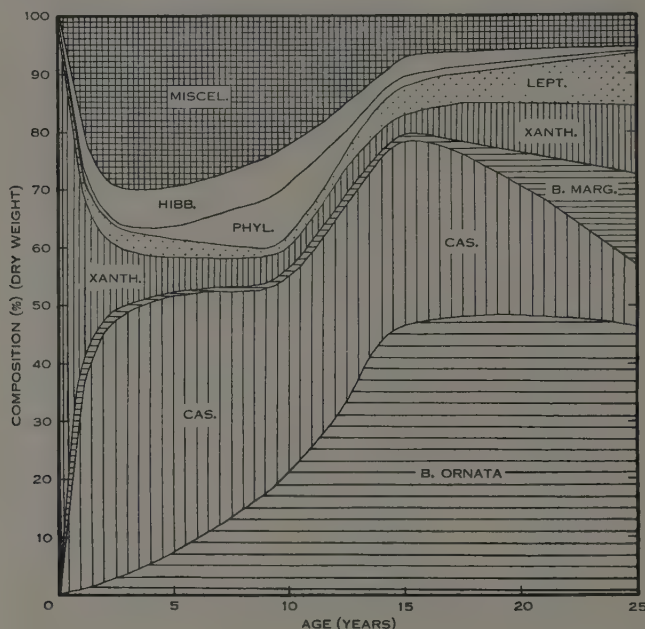


Fig. 8.—Percentage dry weight of the major species and the understorey over a 25-year period after burning.

(xi) *Group E: Miscellany*.—A few specimens of most other sand-plain species (Specht and Rayson 1957a) were collected from one quadrat or another. The dry weight of this group at 2½, 9, 15, and 25 years formed respectively 0·2, 0·6, 0·5, and 0·01 per cent. The annuals, *Centrolepis strigosa* and *Toxanthus muelleri*, were collected only from the 25-year stand and the growth of the moss, *Tortella calycina*, was most extensive in it.

(c) *The Heath Community*

(i) *Species Composition*.—The heath becomes floristically poorer with age. Of 36 species recorded after a fire, only 20 were found after 25 years; five of these 20 contributed less than 1 kg dry weight per acre. Probably only ten of these would persist after 50 years.

The most important species with age (Fig. 8 and Plates 1–3) were briefly as follows: 0–1 year, *Xanthorrhoea*; 1–3 years, *Xanthorrhoea*–*Casuarina*–herb and mat plants; 3–10 years, *Casuarina*–various undershrubs; 10–25 years, *B. ornata*–*Casuarina* with a changing understorey.

The development during the next 25 to 50 years may be predicted. *Xanthorrhoea* and *B. marginata*, which have continuous effective means of vegetative reproduction, will almost certainly become dominants as the original *B. ornata* and *Casuarina* plants die out. *B. ornata*, *Leptospermum*, and Group C and D undershrubs may be re-established from seeds.

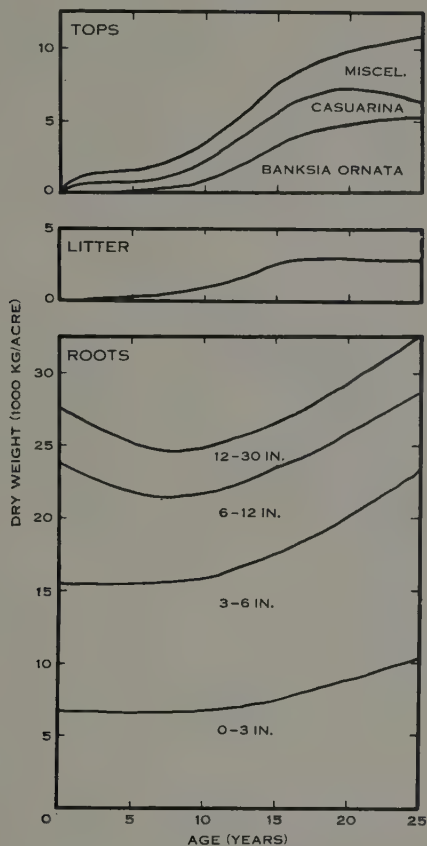


Fig. 9.—Changes in the dry weight of the tops, litter, and roots of the heath with time. Almost 15,000 kg dry weight per acre were found in the tops of the 50-year stand, with *Banksia ornata* contributing 12,920 kg and *Casuarina* 10 kg per acre.

(ii) *Number of Plants.*—All species except *B. marginata*, *Xanthorrhoea*, *Leptospermum*, and *Phyllota pleurandroides* showed a progressive decrease in numbers with the age of the stand.

(iii) *Dry Weight of Vegetation and Litter.*—Figure 9 summarizes the major changes in dry weight which occurred in the aerial organs of heath species during the first 25 years of the pyric succession. As a number of the species regenerated from undamaged root-stocks there was a very rapid increase (mean annual increment of 515 kg dry

weight per acre) in the dry weight of the heath during the first few years after a fire. This was followed by a period during which total growth of tops was slow (mean 240 kg per acre p.a.). A large percentage of the numerous seedlings which followed fire reached their peak and died during this period. Growth of the dominants was slow but continuous.

After about 9 years, the dominant species, *B. ornata*, suddenly increased its rate of growth to produce 3000 kg in dry weight per acre during the next 6 years. This, combined with the steady increase of the other dominants, caused the total dry weight production of all species to rise to 7500 kg per acre at the end of 15 years. Some of this dry weight increase was made at the expense of less important species, for considerable

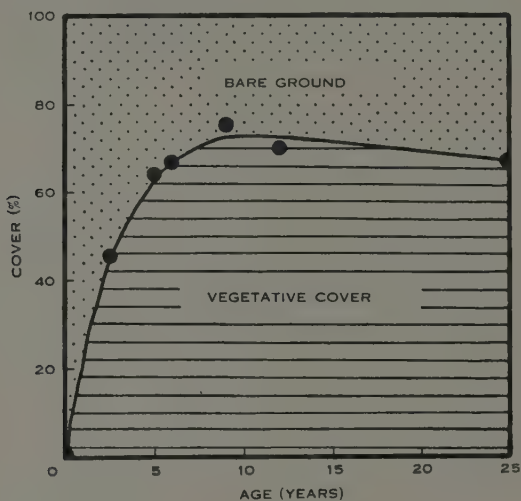


Fig. 10.—Percentage vegetative cover and bare ground over a 25-year period after burning.

degeneration was noticed in these. After this rapid increase in the growth of the stand (780 kg per acre p.a.) the annual growth rate fell continuously to 160 kg per acre towards the 50-year period. Dry weight increase was made largely at the expense of such co-dominant species as *Casuarina*, *Phyllota*, and *Leptospermum* as well as of various miscellaneous species.

Though there was little initial difference in the dry weight of roots, the increase between 9 and 25 years was estimated as 8000 kg/1 ft depth/acre (Specht and Rayson 1957b). This corresponded closely with the difference over the same period of 7500 kg per acre in aerial growth. This was in addition to the 25,000 kg per acre of underground material presumably still living after a fire; hence, at 25 years, the dry weight of the roots was still approximately three times the weight of the tops (Fig. 9).

The quantity of litter to be found in varying stages of decomposition on the ground paralleled the increase in the total dry weight of the tops for much of the period examined (Fig. 9). From 0 to 3 years it was mainly composed of woody skeletons left by the fire. Thereafter the material was composed largely of leaves which had fallen from the major species of the heath, but was continuously supplemented by the dead plants of almost all species. As the ratio of leaf to stem fell with the maturation of many of

Successive layers of the soil were carefully collected from the edge of pits dug under each stand of heath. The A₁ organic horizon was sampled at depths of 0–1 in., 1–3 in., 3–6 in., and 6–12 in. The A₂ horizon was sampled in 1-ft layers, some of which were subsequently bulked when the initial soil analyses revealed little, if any, variation. The top 24 in. of the B horizon was also sampled under the 9-year stand of heath. As several random sites were sampled in each stand, the material from these replicates was later bulked to reduce the laboratory analyses.

Methods used for estimation of the various elements in plant material and in soil were as follows:

carbon, Walkley and Black (1934) (plant and soil); *nitrogen*, Ma and Zuazaga (1942) (plant and soil); *phosphorus*, Zinzadze (1935) and Woods and Mellon (1951) (plant), and Hutton's (unpublished data) method of butyl alcohol extraction followed by colour development by stannous chloride (soil); *potassium*, and also *sodium*, by EEL flame photometer (plant and soil); *calcium*, and also *magnesium*, Cheng and Bray (1951) (plant), and Raupach *et al.* (1954) (soil); *zinc* and *copper*, Jones (1952) (plant and soil); *manganese*, Piper (1944, pp. 346–7) (plant), and Piper (1944, pp. 143–5) (soil); *iron*, Piper (1944, pp. 342–3) (hand ground plant material and soil).

Except for carbon and nitrogen determinations, the analyses of the plant material were all made after a wet digestion by the three acids, sulphuric, nitric, and perchloric, as described by Piper (1944, pp. 272–5). The digestion of the resinous species of the heath proved rather difficult. At least two additions of nitric and sometimes of perchloric acid had to be made to ensure the complete oxidation of the material. Other material, such as *Hibbertia* spp., contained high concentrations of silica (up to 5 per cent.) which was left as a sludge in the digest and on the Kjeldahl flask. To minimize adsorption of other compounds on this sludge, the digest, diluted with distilled water, was boiled gently for some time.

The nitrogen analyses of the plant material were made after digesting the material with concentrated sulphuric acid in the presence of selenium as a catalyst. The carbon was determined after digestion of the material with chromic and sulphuric acids as outlined by Walkley and Black (1934).

Estimates of soil contents of phosphorus, zinc, copper, manganese, and iron were made after extraction of soil with constant-boiling hydrochloric acid. Nitrogen was determined after sulphuric acid–catalyst digestion; carbon after chromic–sulphuric acid digestion. The cations (potassium, sodium, calcium, and magnesium) were determined on an ammonium chloride leachate (Piper 1944, p. 170). As the content of most of these elements was exceedingly low, the digests were concentrated until measurable quantities were obtained.

(b) Results

The nutrient uptake curves for the stands as a whole are summarized in Figures 12–21.* The concentration of the various elements in the soil is indicated in Table 1.

*Detailed information on the numerous analyses of the component species may be obtained on application to the authors.

TABLE I
ANALYSES OF SOIL SAMPLES COLLECTED FROM FOUR AGES OF HEATH VEGETATION

| Age (years) | Depth* (in.) | C (%) | N (%) | C/N Ratio | P (p.p.m.) | K (m-equiv/100g) | Na (m-equiv/100g) | Ca (m-equiv/100g) | Mg (m-equiv/100g) | Zn (p.p.m.) | Cu (p.p.m.) | Mn (p.p.m.) | Fe (%) |
|----------------|-----------------|----------|----------|--------------|---------------|---------------------|----------------------|----------------------|----------------------|----------------|----------------|----------------|-----------|
| 2½ | 0-1 | 0.35 | 0.025 | 14 | 12 | 0.03 | 0.03 | 1.22 | 0.29 | 2.5 | 0.60 | 17.6 | |
| | 1-6 | 0.31 | 0.019 | 16 | 7 | 0.03 | 0.03 | 0.85 | 0.20 | | | 6.7 | |
| | 6-12 | 0.16 | 0.008 | 20 | 5 | 0.02 | 0.03 | 0.41 | 0.15 | | | 5.0 | |
| | 12-30 | 0.05 | 0.004 | 12 | 5 | 0.02 | 0.02 | 0.27 | 0.32 | | | | |
| 9 | 0-1 | 0.60 | 0.031 | 19 | 8 | 0.04 | 0.05 | 1.69 | 0.38 | 2.3 | 0.57 | 22.1 | 0.130 |
| | 1-6 | 0.36 | 0.021 | 17 | 6 | 0.02 | 0.04 | 0.81 | 0.32 | 1.5 | 0.64 | 7.2 | 0.088 |
| | 6-12 | 0.11 | 0.009 | 12 | 4 | 0.02 | 0.03 | 0.44 | 0.09 | 1.1 | 0.40 | 4.9 | 0.075 |
| | 12-30 | 0.05 | 0.005 | 10 | 4 | 0.02 | 0.03 | 0.32 | 0.17 | 1.6 | 0.55 | 4.8 | 0.170 |
| | 30-60 | 0.03 | 0.003 | 10 | 3 | 0.02 | 0.03 | 0.20 | 0.18 | 1.8 | 0.80 | 5.2 | 0.156 |
| | 60-68 | 0.02 | 0.001 | 20 | 2 | 0.02 | 0.03 | 0.20 | 0.13 | 2.6 | 0.85 | 10.8 | 0.098 |
| | 68-92 | 0.05 | 0.007 | 7 | 24 | 0.18 | 0.65 | 1.52 | 3.22 | 12.5 | 0.64 | 17.2 | 2.05 |
| | | | | | | | | | | | | | |
| 15 | 0-1 | 0.54 | 0.026 | 21 | 11 | 0.03 | 0.04 | 1.33 | 0.32 | 2.6 | 0.35 | 19.1 | |
| | 1-6 | 0.39 | 0.021 | 19 | 5 | 0.03 | 0.04 | 0.98 | 0.31 | | | 8.4 | |
| | 6-12 | 0.14 | 0.009 | 16 | 6 | 0.02 | 0.03 | 0.30 | 0.07 | | | 6.2 | |
| | 12-30 | 0.06 | 0.005 | 12 | 4 | 0.02 | 0.03 | 0.30 | 0.18 | | | | |
| 25 | 0-1 | 0.78 | 0.044 | 18 | 18 | 0.07 | 0.14 | 2.90 | 0.92 | 3.6 | 0.17 | 15.6 | |
| | 1-6 | 0.37 | 0.024 | 15 | 10 | 0.05 | 0.06 | 1.40 | 0.34 | | | 6.4 | |
| | 6-12 | 0.16 | 0.011 | 14 | 5 | 0.03 | 0.04 | 0.59 | 0.24 | | | 5.2 | |
| | 12-30 | 0.06 | 0.006 | 10 | 4 | 0.04 | 0.03 | 0.30 | 0.24 | | | | |

* A₁ horizon, 0-12 in., grey sand and organic matter; A₂ horizon, 12-60 in., yellow sand; A₃ horizon, 60-68 in., white sand with red mottlings; B horizon, 68-92 in., orange to grey solonized sandy clay.

(i) *The Tops*.—The shape of the curves for the content of a number of elements (P, K, Mg, and Zn) in the tops, plotted against time, was essentially similar to that shown for dry weight. This was especially so where the percentage concentration of the elements was relatively constant for all major species. Any change in species composition of the stand then did not materially alter the shape of the curve. *Casuarina*, however, showed higher levels of nitrogen (0.75 per cent.) and calcium (1.02 per cent.) than most other species. When this important species began to degenerate in the stand (between the ages of 15 and 25 years), the loss of these two elements tended to flatten off the curves (Figs. 13 and 17).

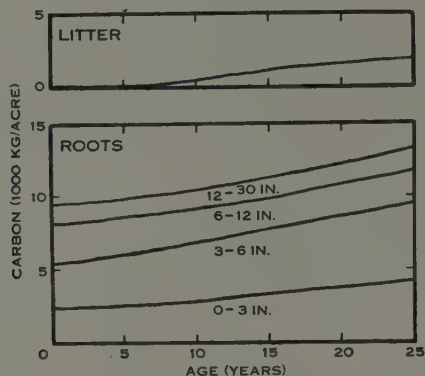


Fig. 12.—Changes in the carbon content of the litter and roots of the heath with time. Composition (C): litter 41–44 per cent.; roots 36–43 per cent.

In the case of nitrogen, the community appeared to hasten its own destruction by excluding the only species capable of fixing atmospheric nitrogen, viz. *Phyllota* and *Casuarina*. *Phyllota*, a legume, has *Rhizobia* associated with its roots; *Casuarina* is reputed to have mycorrhiza on its roots capable of fixing atmospheric nitrogen (Specht and Rayson 1957b). The community must then rely on nitrogen returned as litter or from the occasional thunderstorm.

A decrease in percentage content of an element in any one of the aging major species also produced a similar flattening or depression of its absolute content/time curve. The percentage of sodium in tops of *B. ornata* fell steadily over the first 25 years of the succession, resulting in a marked flattening of the curve during the 15–25 year period (Fig. 16). The percentage content of copper and particularly manganese in *B. ornata* also fell between the 15th and 25th year. This resulted in a marked depression in the curve of absolute content over this period (Figs. 20 and 21). The reduction in percentage mineral content of tops with time probably paralleled the fall in the ratio of leaves to stems, which usually had a lower mineral content than the leaves.

It is difficult to understand why sodium should become limiting in the vegetation, for the Makin sand has a solonized subsoil (0.65 m-equiv. Na/100 g soil). Perhaps the flattening of the curve for sodium was induced by the dearth of another element.

On the other hand, copper and manganese received no additions from external sources. Not only did these elements reach a maximum concentration per acre in the

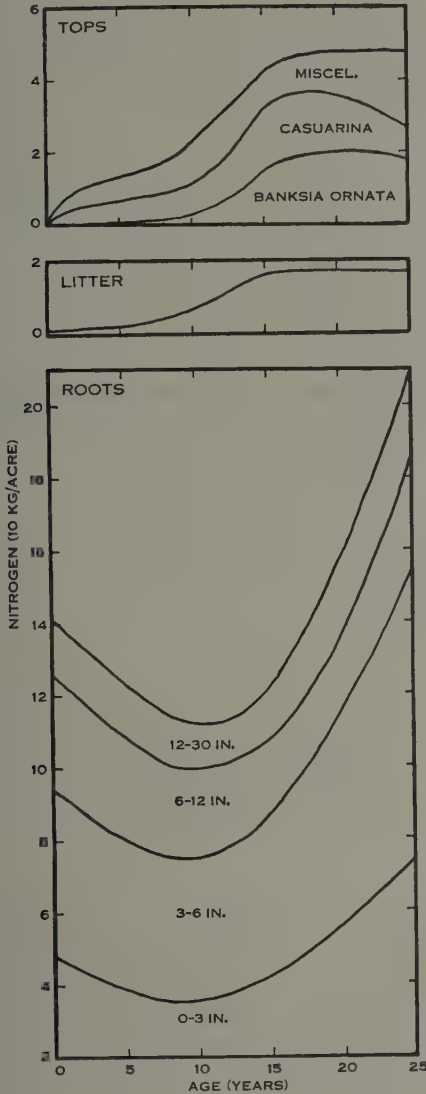


Fig. 13

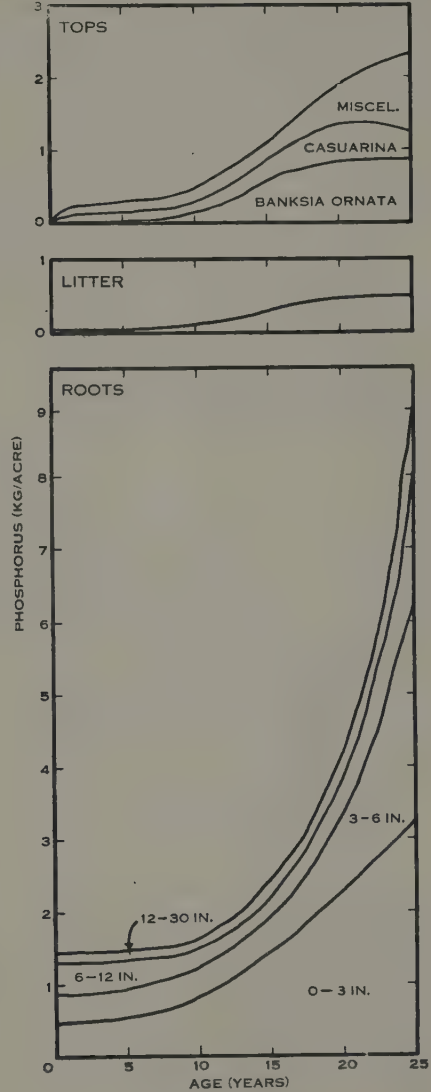


Fig. 14

Fig. 13.—Changes in the nitrogen content of the tops, litter, and roots of the heath with time. Composition (N): tops 0.81 per cent. ($2\frac{1}{2}$ years) to 0.44 per cent. (25 years); litter 0.40–0.71 per cent. Nitrogen content of the tops of 50-year stand: 57,600 g per acre.

Fig. 14.—Changes in the phosphorus content of the tops, litter, and roots of the heath with time. Composition (P): tops 140–210 p.p.m.; litter 80–160 p.p.m.; roots 38–330 p.p.m. Phosphorus content of the tops of 50-year stand: 2000 g per acre.

tops at the 15-year stage, but, particularly in the case of manganese, the level later fell as the element was returned to the soil as litter. A closed cycle for the element from plant to surface and back to plant probably then occurred.

In all, it seemed that several elements (N, Cu, Mn, and possibly Ca and Na), showed negligible increments, or even decrements, in the aerial organs of the heath at approximately the 25-year stage.

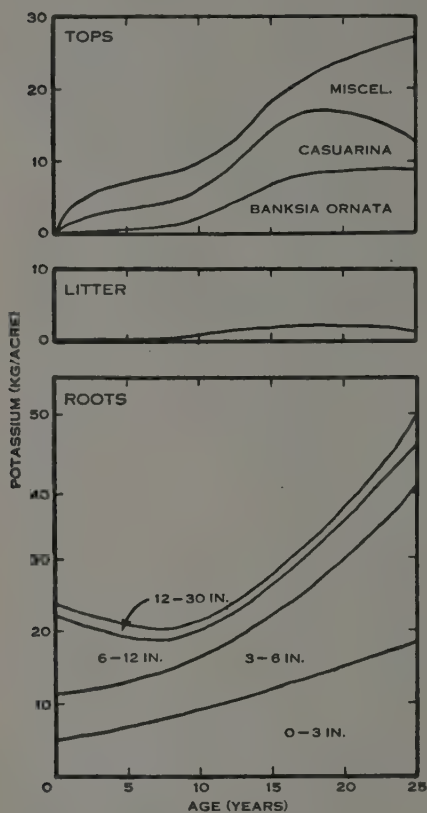


Fig. 15

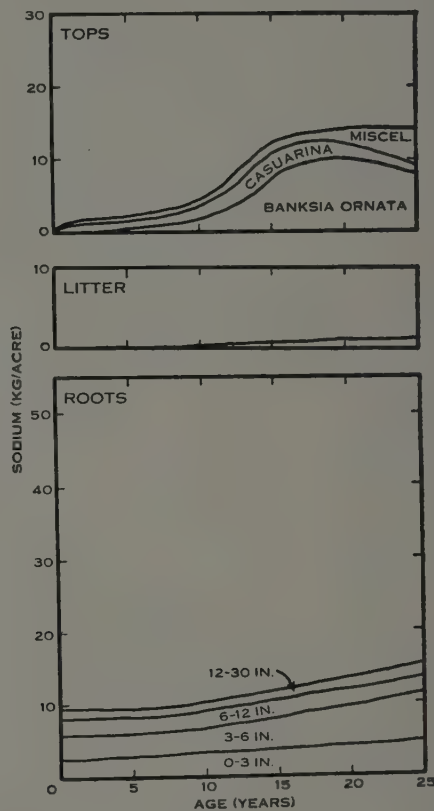


Fig. 16

Fig. 15.—Changes in the potassium content of the tops, litter, and roots of the heath with time. Composition (K): tops 0.406 per cent. (2½ years) to 0.252 per cent. (25 years); litter 0.049–0.069 per cent.; roots 0.052–0.179 per cent. Potassium content of the tops of 50-year stand: 24,500 g per acre.

Fig. 16.—Changes in the sodium content of the tops, litter, and roots of the heath with time. Composition (Na): tops 0.133–0.163 per cent.; litter 0.014–0.041 per cent.; roots 0.032–0.048 per cent. Sodium content of the tops of 50-year stand: 26,900 g per acre.

Unfortunately the authors were unable to locate a stand of heath older than 25 years growing in an environment identical with that of the younger stands at Dark Island. A stand approximately 50 years of age was examined at Meningie (see Section V(a) above) and may serve to indicate some of the further trends in the mineral nutrition of the heath vegetation. The data are presented with many reservations, for the environment (climate, topography, and soil) differed somewhat in many small respects. The results of the analyses are indicated in the captions to Figures 12–21.

Although there was a steady increase in the dry weight of the total heath vegetation from 25 to 50 years, the flattening of the absolute content/time curve was continued for nitrogen and calcium, but not for copper, manganese, and sodium. The soil there may have been slightly richer in copper and manganese than at Dark Island. The leaching of manganese, a very mobile element in these sands, would have been less under the lower rainfall at Meningie than at Dark Island. The soil undoubtedly received considerable cyclic salt (predominantly sodium chloride) from sea spray as well as from old saline lake beds nearby. Dark Island received atmospheric accessions rich in calcium, not sodium—probably dust particles inflated from the surrounding countryside (Hutton 1953). On the other hand the figures indicated that the elements phosphorus, potassium, and zinc, all of which were steadily increasing in quantity in the tops at the 15- to 25-year stage, would probably reach a maximum value during the 25–50 year period.

(ii) *The Litter*.—Most of the curves for content of elements in the litter plotted against time showed slopes similar to those of the litter dry weight/time curve. That for carbon (Fig. 12), increased continuously, while that for manganese (Fig. 21) after reaching a peak at 15 years, fell steadily.

In general, there was a considerable reserve of nutrients available in the litter, especially in the later years of the succession. In the 25-year stand this reserve was usually from one-fifth to one-third of that found in the tops. Calcium values (Fig. 17) were almost as high as one-half of that present in the tops, whereas the more mobile sodium and potassium (Figs. 15 and 16) were as low as one-twelfth and one-eighteenth respectively of that in the tops.

As the C : N ratio of this litter is very high (ranging from 60 at 9 years to 116 at 25 years), microbial activity is low and decomposition of even the less woody fragments of leaf and stem takes from 2 to 3 years. Nevertheless, there is a constant, but slow, return of nutrients to the soil every year, the loss from the litter by decomposition being more than compensated by addition of more litter in the same year.

(iii) *The Roots*.—The amount of most elements in the roots showed a steady increase with time; exceptions were manganese (Fig. 21) where the change in amount was small between 9 and 25 years, and sodium (Fig. 16), where increase in amount in the roots was small. In the latter case, the rate of accumulation in the tops was much greater than in the roots.

Prior to the 9-year stage, the amount of many elements remained relatively constant or fell slightly. However, nitrogen fell steeply over this period, probably owing to the rapid decomposition of those dead fractions containing reasonable quantities of nitrogenous compounds (Fig. 13). Zinc and manganese, on the other hand, steadily increased during this period (Figs. 19 and 21).

(iv) *The Soil*.—Undoubtedly, there must be a marked increase in the nutrient content near the surface of the soil when fire suddenly releases the nutrients accumulated during several years of plant growth. The very rapid regeneration of the heath vegetation in the first years of the pyric succession is greatly aided by this sudden release of minerals as well as by the reduced competition for water (Specht 1957b). By the end of $2\frac{1}{2}$ years, this increment of nutrients was essentially depleted—phosphorus and copper, however, were still comparatively high. The growth of the

heath vegetation slowed down after this initial spurt of growth (Fig. 9). One may assume that at this stage many of the nutrients must come from the deep B horizon of clay which contains a higher concentration of most elements than does the sand.

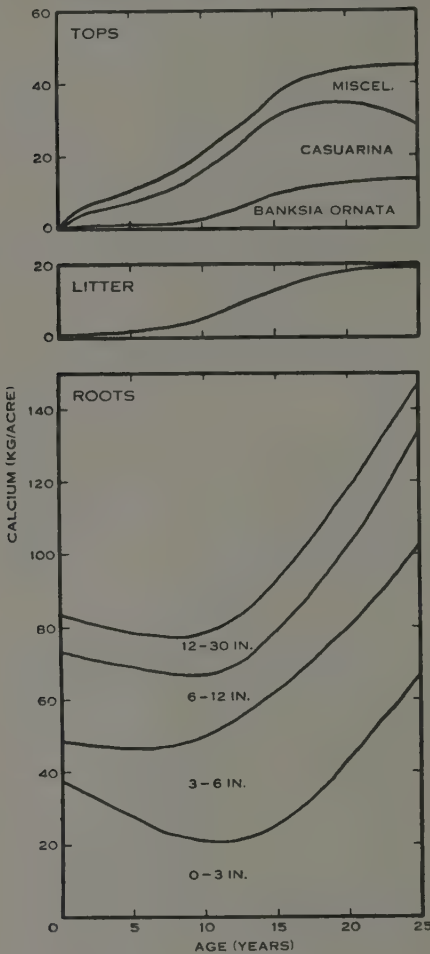


Fig. 17

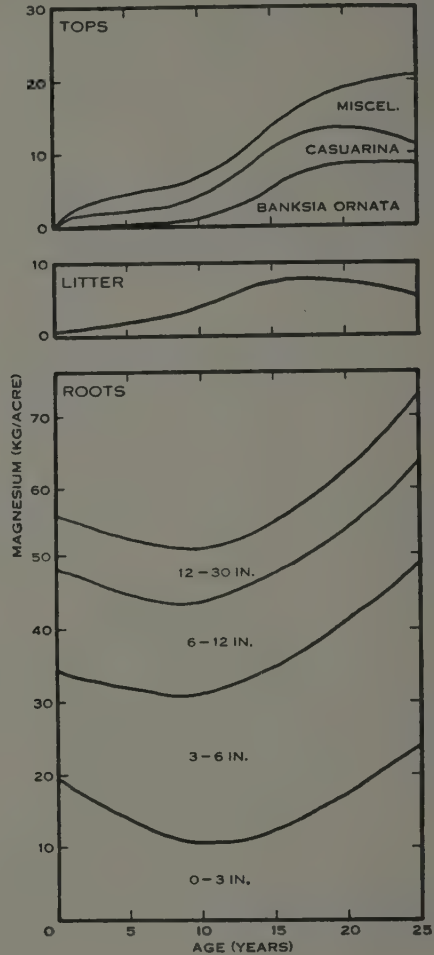


Fig. 18

Fig. 17.—Changes in the calcium content of the tops, litter, and roots of the heath with time. Composition (Ca): tops 0.41–0.65 per cent.; litter 0.40–0.66 per cent.; roots 0.17–0.66 per cent. Calcium content of the tops of 50-year stand: 38,800 g per acre.

Fig. 18.—Changes in the magnesium content of the tops, litter, and roots of the heath with time. Composition (Mg): tops 0.24 per cent. (2½ years) to 0.19 per cent. (25 years); litter 0.18–0.53 per cent.; roots 0.17–0.27 per cent. Magnesium content of the tops of 50-year stand: 28,300 g per acre.

The nitrogen content of the clay, however, is much lower than that of the surface sand, while the calcium, copper, and manganese levels are approximately the same. Atmospheric nitrogen was probably fixed continuously in the surface soil by *Phyllota* and *Casuarina*.

The soil analyses indicated that the nutrients remain relatively constant in the top 30 in. of sand for the next 15 years. There may have been a tendency for a slight increase in nutrient content in the surface layers of the 9-year sample followed by a slight decrease in the 15-year sample, but it was doubtful whether this was significant.

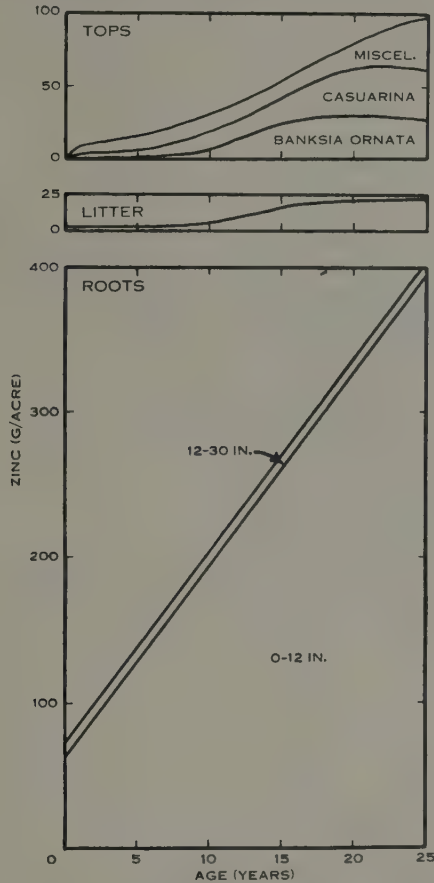


Fig. 19.—Changes in the zinc content of the tops, litter, and roots of the heath with time. Composition (Zn): tops 7.1–9.7 p.p.m.; litter 6.2–9.2 p.p.m.; roots 3.2–13.8 p.p.m. Zinc content of the tops of 50-year stand: 90 g per acre.

If it was significant it was probably associated with the rapid growth between these two times (Fig. 9). However, the 25-year sample showed a marked increase in the concentration of nearly all nutrients in the surface 1 in. and possibly throughout the organic A₁ horizon. The increase in most of the nutrients at this stage was probably associated with the parallel degeneration of two of the co-dominants, *Phyllota* and *Casuarina*, both of which showed higher concentrations of nutrients than most of the other component species of the heath.

Soil copper and manganese were the only exceptions to this rule at the 25-year stage. Both decreased in concentration in the surface soil as the stand aged. In the case of copper, where little difference existed between the levels in the sand and the clay, the steady increase in the amount of copper in the tops and litter over the 25-year period was probably made at the expense of the A_1 horizon. The clay evidently supplied little copper to the vegetation.

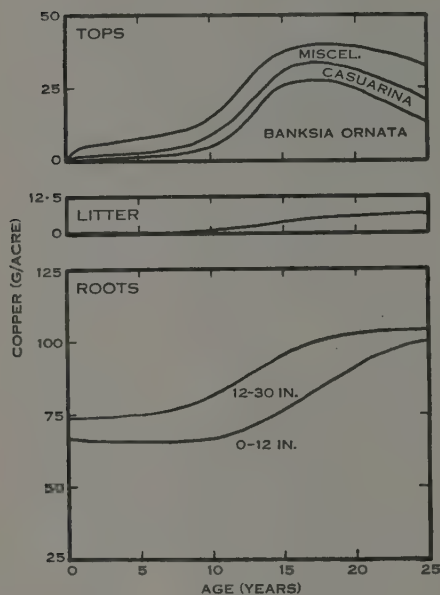


Fig. 20

Fig. 20.—Changes in the copper content of the tops, litter, and roots of the heath with time. Composition (Cu): tops 3.0–4.9 p.p.m.; litter 1.3–2.3 p.p.m.; roots 2.1–3.5 p.p.m. Copper content of the tops of 50-year stand: 47 g per acre.

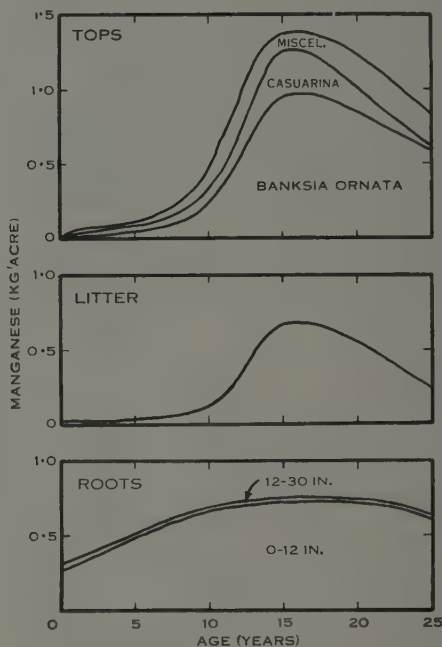


Fig. 21

Fig. 21.—Changes in the manganese content of the tops, litter, and roots of the heath with time. Composition (Mn): tops 50–183 p.p.m.; litter 79–245 p.p.m.; roots 5.1–68 p.p.m. Manganese content of the tops of 50-year stand: 2400 g per acre.

The case of manganese was not so simple. Both species of *Banksia* accumulated this element in their tissues in surprising concentrations (as high as 441 p.p.m. dry weight of total tops). The constant return of manganese in the litter from these two species (recently fallen leaf litter of *B. ornata* contains 648–915 p.p.m. manganese), supplemented to a smaller degree by the other species of the heath, first caused an increase in the amount in the surface soil of the 9-year stand. Thereafter, however, a decrease was apparent. The first decrease was not surprising as the tops and litter, increasing rapidly in dry weight over the period of 9–15 years, accumulated over 2 kg of manganese per acre. Much of this manganese probably came from the A_1 horizon, although some must have been supplied from the clay. After 15 years, however, although the *Banksia* spp. were still actively increasing in dry weight per acre, the relative concentration of this element per unit dry weight of tops decreased. This

situation, produced by an increase in the ratio of stems to leaves, resulted in a decrease in the total manganese content of these species per acre. In all, the manganese content of the tops and litter fell by almost 1 kg per acre. This must have returned to the soil, and yet the manganese content of the surface soil continued to fall. The manganese released from the litter was very mobile and was rapidly leached into the lower layers of sand, for examination of the A_3 horizon, a zone of impeded drainage at 60–68 in., of the 9-year stand indicated that the concentration found there may be double that of the A_2 horizon. This was certainly not the case with the other elements.

VII. DISCUSSION

The development of this heath community after a fire is very similar to regeneration of sclerophyll bush in the homoclines of the Mediterranean coast (Braun-Blanquet 1932), western United States (Cooper 1922, Sampson 1944), and South Africa (Adamson 1935). The most striking similarity is in the high proportion of fire-resistant species in the sclerophyll bush. All authors record an increased number of species after a fire. Adamson (1935) has drawn attention to the comparative paucity of annuals in the southern hemisphere. Numerous annuals persist for 5 years after a fire in chaparral (Sampson 1944)—in heath two annual species occur in the first season only, and even then good opening rains are necessary for their germination.

Early changes in regenerating heath can be attributed to intraspecific and interspecific competition. Soil moisture is conserved by burning (Specht 1957*b*). The stress on vegetation from insufficient water is relieved immediately after a fire and elements normally suppressed by established heath are able to develop. During the drought of the summer of 1950–51 the mortality rate of established bushes on stands 5, 10, and 12 years old was higher than that of young regrowth plants of the same species on a recent burn. The decrease in the number of plants with age has been depicted as a series of smooth curves; in reality the fall-off is stepwise, coinciding with drought conditions. The expected frequency of drought in the area is approximately once in 5 years (Trumble 1948).

Taller shrubs and understorey plants compete for light and space as the heath ages (Fig. 11). Undershrub skeletons have been found under larger bushes and many existing plants in the 25-year stand form a semi-prostrate fringe around larger bushes. This position appears to be adequate for light and more favourable for moisture than the unshaded ground; there is an appreciable difference in surface soil temperatures between shaded and unshaded positions (Specht and Rayson 1957*a*). Similar spatial relations do not hold in underground organs. The horizontal spread of roots is such that densities in the top 12 in. of soil, where most roots are concentrated, are much the same in apparently vegetated and unvegetated positions.

Cooper (1922) has described the effect of fire on the sprouting broad-leaved sclerophyll scrub of the United States as a disruption of the sere, which is followed by a short secondary succession and a rapid reversion to the original scrub form. This description cannot possibly be applied to the heath. The climax community has not been observed in the area. In aging heath, the factor most concerned in the changes among major species is the possible life-span of any plant when it is not subject to apparent external stress. *Banksia ornata* and *B. marginata* bushes have been observed

to senesce and degenerate. A phase difference in the senescence of species which have effective means of survival without a fire would cause a rhythmic change in dominants. A process of regeneration and degeneration, similar to that observed by Watt (1947) would ensue. The continuance of the present widespread form of heath, dominated by *B. ornata*, *Casuarina*, and *Xanthorrhoea* and containing numerous other species, depends on regular burning.

The Makin sand is much poorer than the soils analysed by Hannon (1956) from around Sydney. Her lowest figure for nitrogen is 360 ± 90 p.p.m. whereas only the first inch of the 25-year stand rises above this figure to 440 p.p.m.; at Dark Island most figures range from 10 to 260 p.p.m. Similarly the C : N ratio of 10 : 21 in the Makin sand is lower than that (16:50) found around Sydney. If leaf analyses are of any value, phosphorus is also lower at Dark Island. Beadle (1954) gave values of 160–430 p.p.m. elemental phosphorus in the oven-dried leaves of several species of the dry sclerophyll forest near Sydney. At Dark Island the values range from 90 to 240 p.p.m. in a stand 7 years old (the mean and S.D. of all the leaves from 10 bushes were: *B. ornata*, 239 ± 30 ; *B. marginata*, 224 ± 55 ; *Xanthorrhoea*, 224 ± 31 ; *Casuarina*, 154 ± 42 ; *Phyllota*, 90 ± 15).

In spite of the poverty of the Makin sand, every observation indicated an increase, never a decrease, in the numbers of any species following a fire. This is contrary to the evidence found by Beadle and Burges (1949) for the Sydney area. It was only as the heath aged that numbers markedly decreased, mostly owing to competition for water during the first 20 years of the succession. After that, certain nutrients may become limiting and reduce the uptake of others.

Although a downgrade trend was not obvious in the dry weight of the heath per acre over a period of 50 years, the results indicated that the uptake of several nutrients reached a maximum value in the tops during this period. Nitrogen, phosphorus, potassium, calcium, zinc, and possibly copper, manganese, and sodium may be present in amounts limiting growth in this impoverished soil, the Makin sand, and consequently may induce a degeneration of the heath. Although the results showed that most of these elements may become limiting in the tops with time, possibly only one or two may be the critical elements. The uptake of the others may be controlled by the deficiency of key elements.

Phosphorus was suspected as one of these key elements, for pasture species failed to survive long after germination without its addition. The effect of phosphorus in limiting the growth of some sclerophyllous species of the Hawkesbury sandstone near Sydney was demonstrated in pot-cultures by Beadle (1954), though his results are of doubtful significance statistically. These introductory experiments led to the more critical work of Hannon (1956) who satisfactorily confirmed his indications and showed that, provided phosphorus deficiency was corrected, nitrogen may also be limiting.

There is some doubt whether the same results will be found in the field. In southern California summer drought usually prevented the chaparral from adding the extra growth which was found in the laboratory on either N or N + P treatment (Hellmers, Bonner, and Kelleher 1955). However, as Specht (1953) has shown, highly

significant growth responses have been obtained in the field with phosphorus fertilization. Nitrogen alone showed no response but some significant response may be obtained with phosphorus and nitrogen in combination. These results will be discussed in a later paper in this series.

The increasing growth of the heath is not necessarily associated with an increase in the total content of all elements. It is very likely that the increase in dry weight of the stand is made at the expense of early-maturing shrubs, then of such species as *Casuarina* and *Phyllota*, which accumulate considerable quantities of some elements (nitrogen, calcium) in their tissues. The element is released on the death of these species and is absorbed by the more hardy species which can survive on much lower concentrations. However, this is not always the case. Some species are able to persist and grow on lower concentrations of an element as the plant ages. Perhaps there is a luxury uptake of these elements in the early stages of the pyric succession followed by a dilution of the element with the increased dry weight production which accompanies age.

In spite of these trends in the tops, the roots showed steadily increasing concentrations of most elements. The availability of these elements will be investigated at a later date. In the case of phosphorus, the work of Russell and Martin (1953) indicated that a considerable quantity of the phosphorus absorbed from a weak phosphorus medium was bound in the roots of barley plants; only a small percentage was translocated to the tops. One may suspect a similar situation in the heath not only with phosphorus but with other elements which have to satisfy metabolic sites in the roots before translocation can occur. Paralleling the increase in litter, the nutrient content of the soil increased with the age of the stand. Perhaps this favours slightly greater translocation of the elements from roots to tops in older stands.

As the heath aged increasing amounts of most nutrients were found in the fruits of *Banksia* and in the dead leaves of *Xanthorrhoea* held some distance above the ground, the site of greatest microbial activity. At 25 years one-fourth to one-third of the total nutrients in the tops were found in these organs; only potassium and calcium showed lower concentrations of one-sixth and one-fifth respectively. By 50 years the amount bound in these organs rose to over 50 per cent. As well, considerable amounts of nutrients were bound in the litter. This accumulation was made at the expense of the actively growing organs.

Degradation of the stand must inevitably occur to restore much of the "working capital" of nutrients so bound to the ecosystem.

VIII. ACKNOWLEDGMENTS

The authors wish to thank Professor J. G. Wood for advice throughout this project and Mrs. Jean Bird and Miss Helene Martin for assistance with the harvests.

IX. REFERENCES

- ADAMSON, R. S. (1935).—*J. Ecol.* **23**: 44–55.
BEADLE, N. C. W. (1954).—*Ecology* **35**: 370–5.
BEADLE, N. C. W., and BURGESS, A. (1949).—*Aust. J. Sci.* **11**: 207–8.
BRAUN-BLANQUET, J. (1932).—"Plant Sociology." (McGraw-Hill Book Co.: New York.)
CANFIELD, R. H. (1941).—*J. For.* **39**: 388–94.

- CANFIELD, R. H. (1944).—*J. For.* **42**: 192-4.
- CHENG, K. L., and BRAY, R. H. (1951).—*Soil Sci.* **72**: 449-58.
- COALDRAKE, J. E. (1951).—C.S.I.R.O. Aust. Bull. No. 266.
- COOPER, W. S. (1922).—Carnegie Inst. Wash. Publ. No. 319.
- HANNON, NOLA J. (1956).—*Proc. Linn. Soc. N.S.W.* **81**: 119-43.
- HELLMERS, H., BONNER, J. F., and KELLEHER, J. M. (1955).—*Soil Sci.* **80**: 189-97.
- HUTTON, J. T. (1953).—Atmospheric accessions. Aust. Conf. Soil Sci., Adelaide 1953, Paper No. 6. 11. 1.
- JONES, G. B. (1952).—*Anal. Chim. Acta* **7**: 578-84.
- MA, T. S., and ZUAZAGA, G. (1942).—*Ind. Eng. Chem. (Anal.)* **14**: 280-2.
- PARKER, K. W., and SAVAGE, D. A. (1944).—*J. Amer. Soc. Agron.* **36**: 97-110.
- PIPER, C. S. (1944).—"Soil and Plant Analysis." (Waite Agric. Res. Inst.: Adelaide.)
- RAUPACH, M., STACE, H. C. T., TUCKER, B. M., BOND, R. D., and HUTTON, J. T. (1954).—The titration of calcium and magnesium by E.D.T.A. C.S.I.R.O. Aust. Div. Soils., Div. Rep. 9/54.
- RAYSON, PATRICIA (1957).—*Aust. J. Bot.* **5**: 86-102.
- ROE, R. (1947).—Coun. Sci. Industr. Res. Aust. Bull. No. 210.
- RUSSELL, R. S. and MARTIN, R. P. (1953).—*J. Expt. Bot.* **4**: 108-27.
- SAMPSON, A. W. (1944).—Agric. Expt. Stat. Berkeley Calif. Bull. No. 685.
- SPECHT, R. L. (1953).—Ph. D. Thesis, University of Adelaide.
- SPECHT, R. L. (1957a).—*Aust. J. Bot.* **5**: 137-50.
- SPECHT, R. L. (1957b).—*Aust. J. Bot.* **5**: 151-72.
- SPECHT, R. L., and RAYSON, PATRICIA (1957a).—*Aust. J. Bot.* **5**: 52-85.
- SPECHT, R. L., and RAYSON, PATRICIA (1957b).—*Aust. J. Bot.* **5**: 103-14.
- TRUMBLE, H. C. (1948).—*J. Dep. Agric. S. Aust.* **52**: 55-64.
- WALKLEY, A., and BLACK, I. A. (1934).—*Soil. Sci.* **37**: 29-38.
- WATT, A. S. (1947).—*J. Ecol.* **35**: 1-22.
- WOODS, J. T., and MELLON, M. G. (1941).—*Ind. Eng. Chem. (Anal.)* **13**: 760-4.
- ZINZADZE, CH. (1935).—*Ind. Eng. Chem. (Anal.)* **7**: 227-30.

DARK ISLAND HEATH. VI



Fig. 1.—A stand of heath photographed 1 month after a fire; skeletons of *Banksia ornata* and *Casuarina pusilla*, and regenerating *Xanthorrhoea australis*, already 12–18 in. high, are shown.
 Fig. 2.—Flowering *Xanthorrhoea australis* on a slight sand ridge which was burnt 9 months before the photograph was taken.

DARK ISLAND HEATH. VI



Fig. 1.—*Banksia ornata* seedlings, 2 in. tall, in a stand of heath burnt 15 months before the photograph was taken.

Fig. 2.—A stand of heath which was fired 12 years before the photograph was taken. The large bushes of *Banksia ornata* and *Xanthorrhoea australis* are prominent, with *Banksia marginata*, *Casuarina pusilla*, and *Phyllota* spp. in the understorey.

DARK ISLAND HEATH. VI



Fig. 1.—A stand of heath 25 years old. *Banksia ornata* and *Xanthorrhoea australis* are dominant with *Leptospermum myrsinoides* and *Banksia marginata*.

Fig. 2.—A stand of heath about 50 years old. Very large bushes of *Banksia ornata* and clumps of *Xanthorrhoea australis* are shown with a mat of *Kunzea pomifera* covering the ground.

DARK ISLAND HEATH. VI



Fig. 1.—A degenerating *Banksia ornata* bush about 50 years old.

Fig. 2.—A thicket of *Banksia marginata* suckers surrounding a dead parent plant in heath 40–50 years since burning.

CHANGES IN CELL WALL ORGANIZATION RESULTING FROM SURFACE GROWTH IN PARENCHYMA OF OAT COLEOPTILES

By A. B. WARDROP* and J. CRONSHAW*

[Manuscript received November 22, 1957]

Summary

The primary walls present during the phase of extension growth in oat coleoptiles possess an almost transverse microfibril orientation on their inner surfaces but on the outer surface the microfibrils are considerably disoriented from this direction, which is consistent with the concept of multi-net mechanism of growth. Coleoptile segments grown at 2°C to depress cell wall formation show no difference in orientation on their inner and outer surfaces; this is also considered to be consistent with the multi-net mechanism. It is shown that the longitudinal ribs of microfibrils present at the cell corners, and hitherto referred to as secondary thickening, are on the outer surface of the cell wall and are considered to arise from a disorientation of microfibrils as a result of multi-net growth. As a result of this microfibril disorientation there is a tendency for the pit fields to be reduced in area.

After surface growth has ceased a secondary wall is formed with a well-defined helical organization distinctly different from that of the primary wall. The implications of these results in terms of previous investigations are discussed.

I. INTRODUCTION

Various concepts have been proposed as to the manner of participation of the cell wall in surface growth of plant cells. In many cases where detailed studies have been made, as on cotton and other seed hairs (Roelofsen 1951; Roelofsen and Houwink 1953), stellate pith cells of *Juncus* (Houwink and Roelofsen 1954), and parenchyma of oat coleoptiles and onion root (Wardrop 1956, Setterfield and Bayley 1957), the evidence has been consistent with the concept of multi-net growth proposed by Houwink and Roelofsen (1954).

This concept is based on the observation that there exists a difference in orientation of the cellulose microfibrils constituting the cell wall framework, between the inner and outer surfaces of enlarging cells. On the inner surface the microfibrils are almost transverse to the major morphological cell axis, whereas on the outer surface they are considerably disoriented from this arrangement. It must thus be envisaged that between these extremes of orientation there exist transitional orientations through the thickness of the cell wall.

Clearly the multi-net mechanism involves relative movement between the microfibrils during surface growth of the cell and further, the final orientation on the outer surface will reflect the extent and polarity of the growth which has taken place. Experimental verification of the latter conclusion was presented by Wardrop (1956).

However, the apparent uniformity of cellulose synthesis over the surface of extending cells of coleoptile parenchyma does raise the additional question of the

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nature of the structural changes which take place during surface growth in the cell wall, i.e. in the region of plasmodesmata and pit fields.

The nature of these changes is also of interest in relation to the earlier concept of mosaic growth proposed by Frey-Wyssling and Stecher (1951) and Stecher (1952). On this view cellulose synthesis was considered to proceed at points where the cytoplasm transiently penetrated the cell wall. Synthesis of cytoplasm was considered to take place at these points, enlarging the wall surface by pushing apart the microfibrils, and the enlarged (thin) areas of the cell wall were considered then to be filled in by the synthesis of new cellulose microfibrils. This idea was based on the observation of thin areas in the cell wall in which microfibrils were less densely packed or absent and of similar areas apparently partially filled in with microfibrils. It was shown subsequently (Wardrop 1955) that the thin areas correspond to points of penetration of the cell wall by plasmodesmata, or to the pit fields.

Although the concept of mosaic growth does not appear tenable it will be clear if multi-net growth does take place then changes in dimensions of the plasmodesmata and pit fields must be capable of explanation in terms of this mechanism.

In addition to the apparent filling-in of the pit fields observed by Frey-Wyssling and Stecher (1951), it was considered that the pit fields tended to be covered by secondary thickening as the cells mature. Thus Muhlethaler (1950) observed at the cell corners longitudinal ribs of cellulose in which the microfibrils run parallel to the cell axis. From Muhlethaler's photographs these ribs become less obvious as the cell matures and this was considered by Muhlethaler to be due to the progressive lateral elaboration of the corner ribs to cover the whole wall and to constitute the secondary cell wall thickening of the coleoptile parenchyma. It may be noted, however, that some of this thickening is present during extension growth, whereas in general secondary thickening does not take place until extension growth has ceased, at least locally within the cell.

In view of these considerations a further study has been made to determine the structural changes taking place in the primary wall of extending coleoptile parenchyma as well as the nature of secondary thickening in mature cells.

II. MATERIAL AND METHODS

The material used consisted of coleoptiles from *Avena sativa* L. (var. Algeribee), which were germinated and grown in the manner previously described (Wardrop 1955). Non-epidermal parenchyma was examined from coleoptiles 2 mm and 5 mm long (i.e. before the end of the phase of cell division) as well as from coleoptiles 1–2 cm long and from mature coleoptiles. Further details are given below. The coleoptiles were macerated by alternate treatments with dilute alkali and dilute acid as in previous studies (Wardrop 1955). The macerated material, which consisted of a suspension of whole cells, was examined, after uranium shadow casting, using an RCA type EMT electron microscope.

Examination in polarized light was made after staining with 1 per cent. congo red in slightly alkaline aqueous solution. X-ray diffraction diagrams of coleoptile parenchyma were prepared from two composite specimens consisting of eight coleoptiles 1.5 and 5 cm in length respectively. The epidermis was peeled off after

discarding the terminal 5 mm and the remaining parenchyma was extracted at 95°C for 4 hours with 1 per cent. hydrochloric acid. After washing free from acid a composite specimen of each group was prepared in the manner described for conifer cambium by Preston and Wardrop (1949). The acid extraction causes some crystallization of the cellulose but does not greatly alter the microfibril orientation in the cell walls (Preston and Wardrop 1949; Wardrop 1949).

III. OBSERVATIONS AND DISCUSSION

In the present study an attempt was made to obtain additional experimental evidence confirming the operation of multi-net growth from the examination of cells taken from the subterminal 5 mm of coleoptiles 2 cm long which had been allowed to extend in auxin (1 mg/l.) at low temperatures. It has previously been shown by Bonner (1935) that coleoptile segments grown at 2°C show no increase in dry weight, so that the processes of cell wall formation and cell extension are independent. In terms of the multi-net concept it might thus be anticipated that if cell wall formation were suppressed extension growth should lead to a disappearance of the transversely oriented microfibrils on the inner cell wall surface.

Control segments showed the usual uniform transverse microfibril orientation on the inner surface (Plate 1, Fig. 1). After extension at 2–4°C, however, similar segments showed no uniform transverse microfibril orientation on either the inner or outer surfaces, the whole wall giving the impression of having been stretched (Plate 1, Fig. 2). This provides further proof of the operation of the multi-net mechanism. In relation to these observations it is of interest that the contraction on plasmolysis of coleoptile segments grown at 4°C is nearly twice that of segments grown at 13°C or 25°C when cell wall formation proceeds normally (Wardrop 1955).

The existence of longitudinal bands of thickening at the cell corners has been referred to by Muhlethaler (1950), Wardrop (1955, 1956), and Setterfield and Bayley (1957). These bands (see Plate 4, Fig. 2) have previously been regarded as secondary thickening, even though they are present throughout the period of extension of the parenchyma. Wuhrmann-Meyer and Wuhrmann-Meyer (1939) described marked changes in the optical anisotropy of maturing parenchyma and subsequently this appears to have been tacitly accepted as arising from a further elaboration of the longitudinal bands already present.

With these points in mind it was considered necessary to examine the nature of the initial corner thickening bands and of the secondary thickening in maturing parenchyma with a view not only to elucidate the structural changes associated with multi-net growth, but to define more exactly the sequence of wall development in the cell ontogeny.

In very young parenchyma from coleoptiles (2–3 mm in length) the bands were absent or poorly developed when viewed in the polarizing microscope. In the electron microscope, however, microfibrils approaching longitudinal orientation could be detected (Plate 2, Figs. 1 and 2). These microfibrils appeared to be on the outer wall surface and not the inner surface as would be expected if they represented secondary thickening. Furthermore, examination of published electron micrographs (see Wardrop (1955), Plate 5, Fig. 1) showed that even in mature parenchyma these bands also appear to be on the outer surface of the cells.

Further examination between crossed nicols of the early stages of extension of the parenchyma showed that the bands became at first more apparent (cells from coleoptiles 1-1.5 cm) and subsequently less apparent in successive maturing stages of the cells. Electron microscopic examination showed that the longitudinally arranged microfibrils appeared to be pulled around the pit fields, gradually encroaching over them, presumably as the result of withdrawal of the cytoplasm of the plasmodesmata (Plate 4, Fig. 1). It can be seen from Plate 2, Figure 1 that in the shorter cells the microfibrils are less perfectly aligned in the longitudinal direction than in the more elongated cells (Plate 3, Fig. 1), which is consistent with the idea that the orientation within the bands results from the growth which takes place.

It will be recalled also that these bands of microfibrils can be detected in autoradiographs (Wardrop 1956). From examination of Plates 2, 3, and 4, it is obvious that because the longitudinally oriented bands of microfibrils are on the outer surface of the cells they do not constitute secondary thickening.

These observations raise the question whether the existence of longitudinally oriented microfibrils on the outer surface of the cell arises as a consequence of multi-net growth or whether their orientation reflects some different condition at the cell corners. In relation to the extending parenchyma of internodes from *Elodea canadensis* this second possibility was discussed by Wilson (1957), who pointed out that if there existed a difference in the mechanism of growth in walls abutting in intercellular spaces and those in contact with adjacent cells, this could result in a difference in microfibril arrangement between the two regions. Although no local difference in structure was detected by Wilson, it is clear that in the material here studied such a difference does exist. Two possibilities may be considered. It may be that the longitudinal microfibrils represent the vestiges of corner thickenings of the cells distorted by subsequent extension growth. On the other hand, in view of the relatively great extension which occurs and the number of these microfibrils present, some may have arisen by disorientation of microfibrils from adjacent regions of the wall. Tentatively, it is proposed to consider this latter view in relation to the multi-net mechanism. The presence of plasmodesmata and pit fields in the walls of expanding cells implies that cellulose deposition cannot proceed evenly over the wall surface, and will be interrupted in those regions where these structures are present. On the other hand, the cell corners are in general free of plasmodesmata. If the microfibrils are deposited in an initially transverse orientation, as proposed by Roelofsen and Houwink (1954), then as extension proceeds disorientation of the microfibrils at the cell corners will be unimpeded. Disorientation of those near plasmodesmata and pit fields, however, will be impeded by these structures. Furthermore, deposition of cellulose will begin earlier in those regions of the wall where plasmodesmata and pit fields are absent, and it may be noted from the work of Meeuse (1941), of Whaley, Mericle, and Heimsch (1952), and Scott *et al.* (1956), that the relative area of cell contact occupied by the cell wall as distinct from intercytoplasmic strands increases as the cells mature. On this view disorientation of the microfibrils from the transverse orientation could proceed to a greater extent at the corners of the cell and in areas free of plasmodesmata and these regions would be thicker than other regions of wall. Furthermore, where the plasmodesmata finally retract from the wall the disorienting

microfibrils will tend to cover the pit field areas and so could give rise to the appearance of the microfibrils encroaching on these regions (Plate 2, Fig. 2; Plate 4, Fig. 1).

The longitudinal microfibrils on the outer surface of the cell would thus arise as a consequence of the continued deposition and disorientation of microfibrils in this region, an interpretation consistent with the concept of Meeuse (1941) that there is a progressive withdrawal of the cytoplasm from the wall as the cell matures. The progressive elimination of the pit fields by the above mechanism also suggests that they do not have any special function in cellulose synthesis as envisaged in the concept of mosaic growth (Frey-Wyssling and Stecher 1951; Stecher 1952; Wardrop 1954).

The changes are seen in exaggerated form in cells which have undergone extension under conditions such that no new cell wall material was formed. When examined in the electron microscope cells grown between 2 and 4°C showed considerable disorientation of the microfibrils on their outer surface (Plate 3, Figs. 1 and 2). Of particular interest, however, is the fact that the pit field (Plate 3, Fig. 2) is completely covered with longitudinally oriented microfibrils.

In terms of the above observations, it can be seen that the longitudinally arranged bands of microfibrils unquestionably represent part of the primary wall (i.e., the membrane present during extension growth) and their origin can be explained at least in principle in terms of the multi-net mechanism. It is of interest in this context to speculate whether the longitudinally oriented thickening on the outer wall of epidermal parenchyma may be similar in origin to the corner thickenings of the subepidermal parenchyma. It may be noted that the corner thickening of subepidermal parenchyma, and the outer wall thickening of epidermal parenchyma, can be seen in autoradiographs of segments cultivated in labelled glucose (Wardrop 1956).

However, as previously stated, the longitudinal bands become less apparent in the polarizing microscope as the cell matures, and it is apparent that this change is associated with some further elaboration of the cell wall structure, which in view of the work of Wuhrmann-Meyer and Wuhrmann-Meyer (1939) might be a deposition of further cellulose layers on the inner surface of the cell wall, i.e., true secondary thickening. Accordingly, an examination was made of parenchyma from coleoptiles in the later stages of maturation.

This examination revealed that in the mature cells there exists, in addition to the primary wall, a secondary wall of helical organization. The texture of this structure is illustrated in Plates 5 and 6. When examined between crossed nicols after staining with congo red, the helical organization of the secondary wall can be seen (Plate 4, Figs. 3 and 4). What is possibly an early stage of the development of this structure can be seen in Plate 5, Figure 1, and its texture at maturity is shown in the electron micrographs of Plate 6. In Plate 6, Figure 2, the secondary wall appeared to have a crossed fibrillar organization, but this must await further investigation.

X-ray diffraction evidence of the development of the secondary wall is seen in the diffraction diagrams of composite samples (Plate 5, Figs. 2 and 3, corresponding to coleoptiles 1.5 and 5 cm long respectively). In Plate 5, Figure 2, only two meridional arcs corresponding to the 002 planes (3.9 Å spacing) can be seen. The spread of the arcs would indicate considerable dispersion of the microfibrils about a predominant transverse orientation. This would be anticipated from the organization of the cell

wall revealed in the electron microscope. In Plate 5, Figure 3 the meridional arcs are largely masked and two strong equatorial arcs are present corresponding to the helical secondary thickening shown in Plate 4, Figures 3 and 4, and in Plates 5 and 6.

In relation to the above observations it can be seen that the deposition of a helical layer in which the microfibrils are oriented at an angle of from 30 to 60° to the major cell axis within the primary wall would tend, when viewed between crossed nicols, to make the wall more isotropic and the contrast of the longitudinal bands would be greatly reduced. Furthermore, once the existence of a helically organized secondary wall is recognized the work of Preston (1938) acquires an added significance in that his demonstration of a relation between cell dimensions and the measured major extinction position of the cells clearly appears to apply to the secondary thickening and so is in agreement with results for other secondary walls.

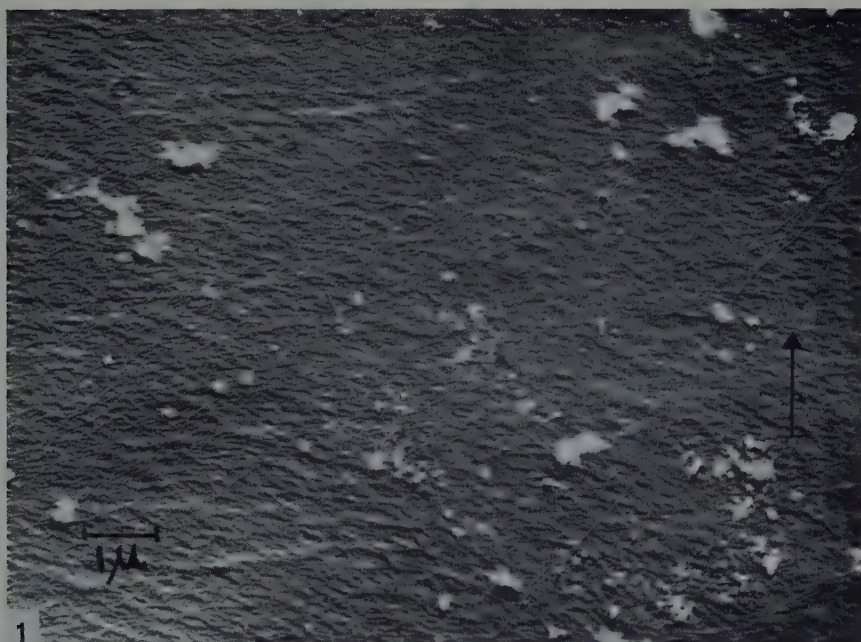
It would also seem that the observations of Muhlethaler (1950) on which he based his concept of tip growth of coleoptiles and parenchyma in part at least represented observations on cells in which secondary thickening had commenced as shown, for example, in his Figure 7 (*loc. cit.*).

To recapitulate, it can be seen that the foregoing discussion covers three aspects of cell wall growth. Firstly, a study of extension growth of coleoptile segments at low temperatures provides further evidence in support of the view that multi-net growth is operative in *Avena* coleoptile parenchyma. Secondly, the longitudinal corner thickenings of parenchyma cells, previously regarded as secondary thickening, must be regarded as part of the primary wall present during the phase of extension growth and, finally, the true secondary thickening possesses a helical organization, and is developed after a phase of extension growth has ceased.

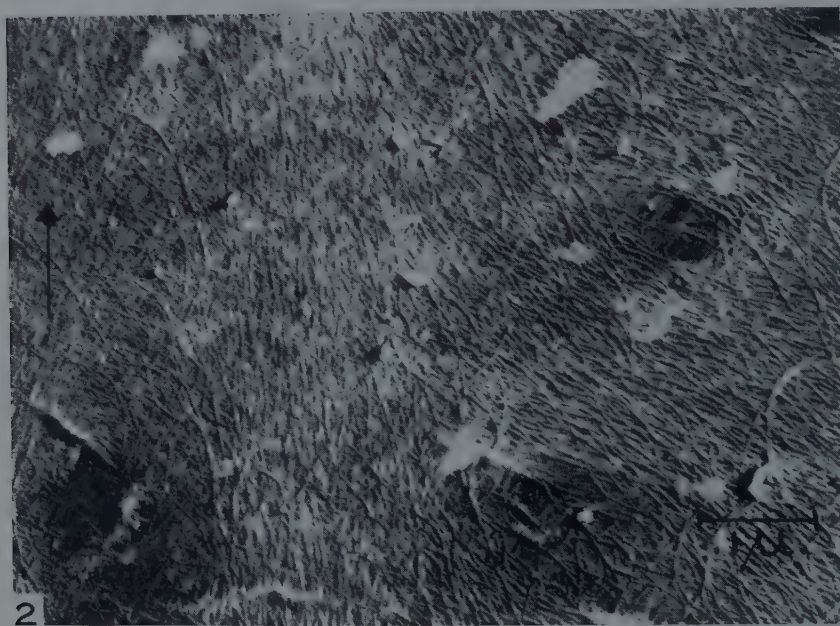
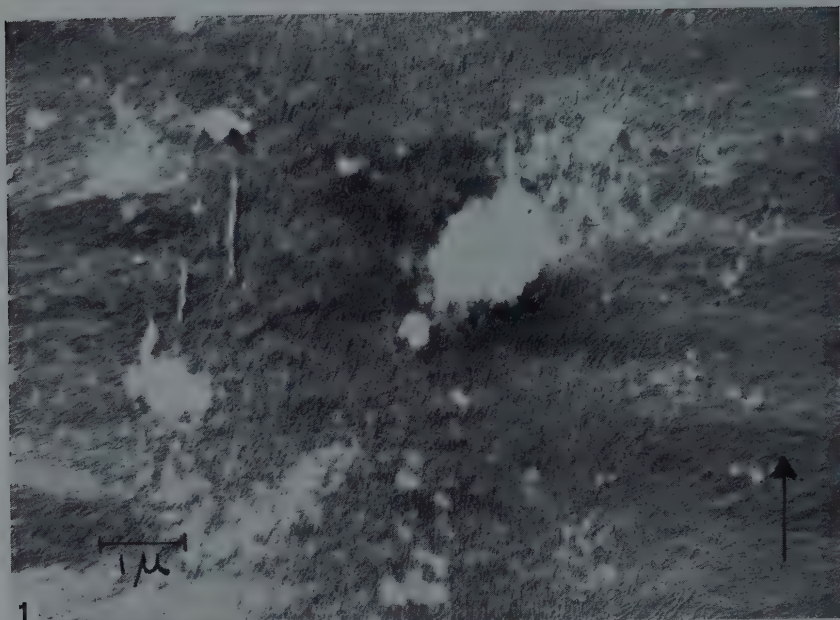
IV. REFERENCES

- BONNER, J. (1935).—*Jb. wiss. Bot.* **82**: 377.
 FREY-WYSSLING, A., and STECHER, H. (1951).—*Experientia* **7**: 420.
 HOUWINK, A. L., and ROELOFSEN, P. A. (1954).—*Acta Bot. Neerl.* **3**: 385.
 MEEUSE, A. D. J. (1941).—*Bot. Rev.* **7**: 249.
 MUHLETHALER, K. (1950).—*Ber. schweiz. bot. Ges.* **60**: 614.
 PRESTON, R. D. (1938).—*Proc. Roy. Soc. B* **125**: 372.
 PRESTON, R. D. and WARDROP, A. B. (1949).—*Biochim. Biophys. Acta* **3**: 549.
 ROELOFSEN, P. A. (1951).—*Biochim. Biophys. Acta* **7**: 43.
 ROELOFSEN, P. A., and HOUWINK, A. L. (1953).—*Acta Bot. Neerl.* **2**: 219.
 SCOTT, F. M., HAMNER, K. C., BAKER, E., and BOWKER, E. (1956).—*Amer. J. Bot.* **43**: 313.
 SETTERFIELD, G., and BAYLEY, S. T. (1957).—*Canad. J. Bot.* **35**: 435.
 STECHER, H. (1952).—*Mikroskopie* **7**: 30.
 WARDROP, A. B. (1949).—*Nature* **164**: 336.
 WARDROP, A. B. (1954).—*Aust. J. Bot.* **2**: 165.
 WARDROP, A. B. (1955).—*Aust. J. Bot.* **3**: 127.
 WARDROP, A. B. (1956).—*Aust. J. Bot.* **4**: 193.
 WHALEY, W. G., MERICLE, L. W., and HEIMSCH, C. (1952).—*Amer. J. Bot.* **39**: 20.
 WILSON, J. K. (1957).—*Ann. Bot.* **21**: 1.
 WUHRMANN-MEYER, K., and WUHRMANN-MEYER, M. (1939).—*Jb. wiss. Bot.* **87**: 642.

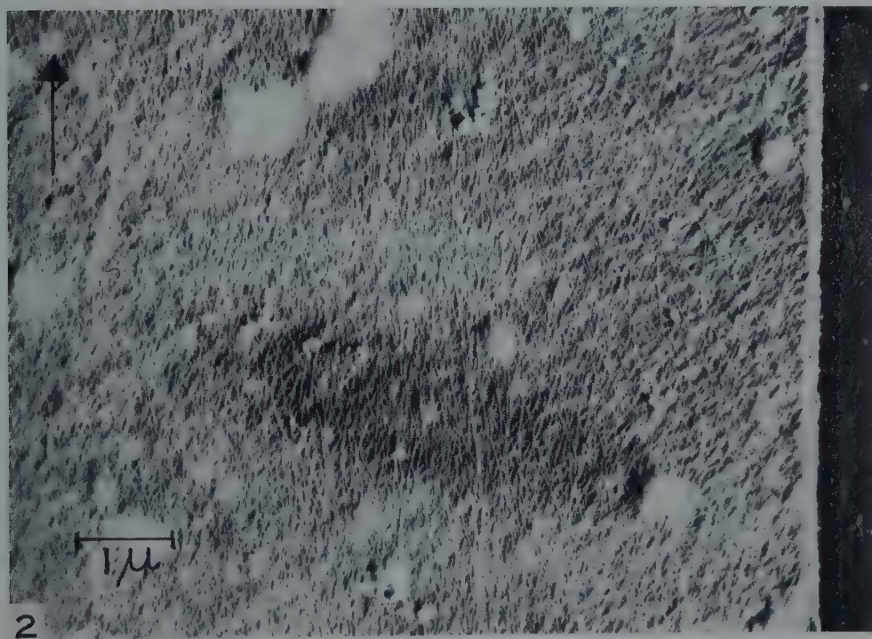
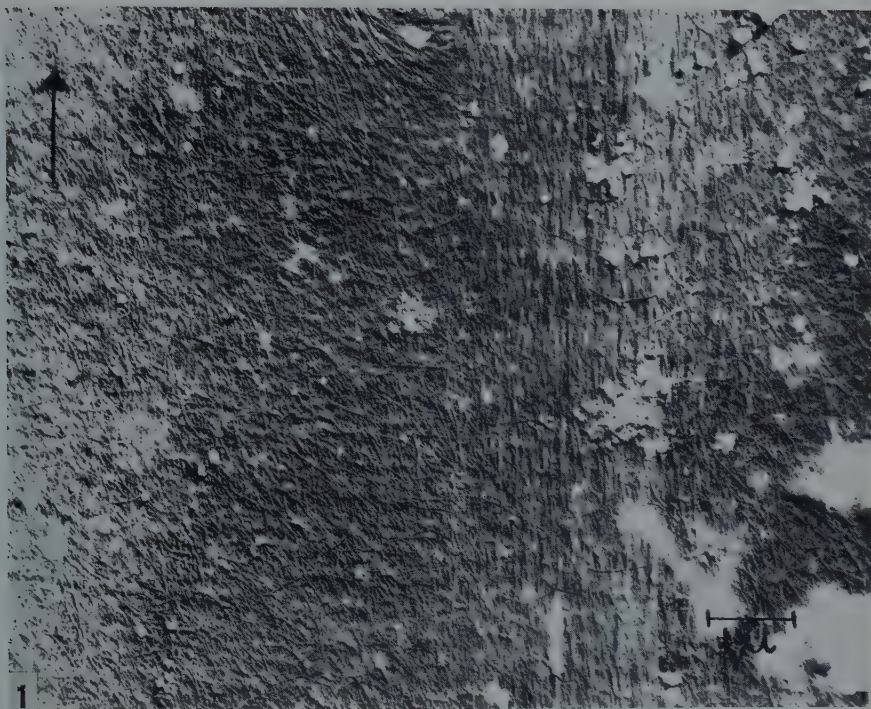
SURFACE GROWTH IN PARENCHYMA OF OAT COLEOPTILES



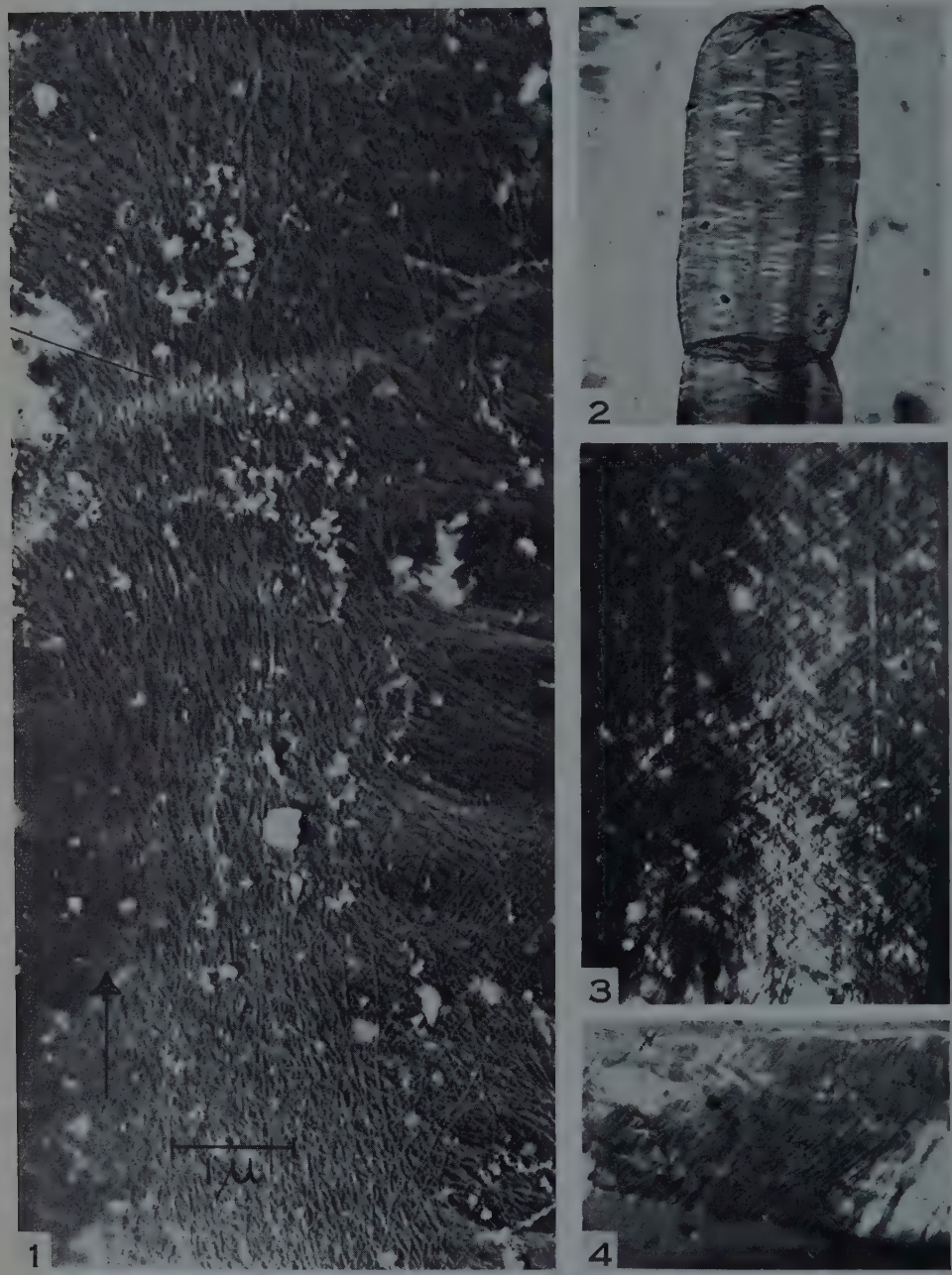
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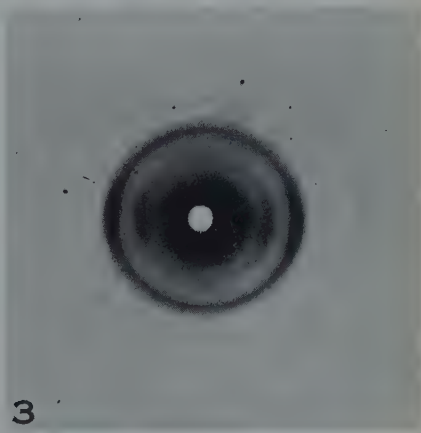
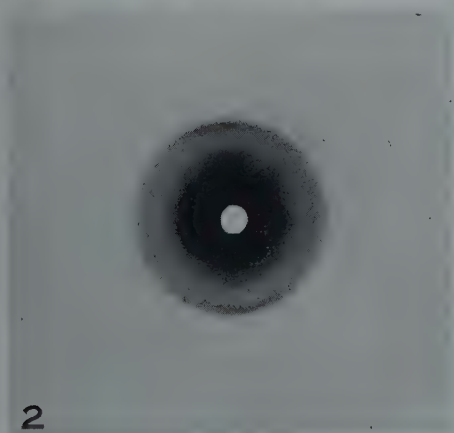
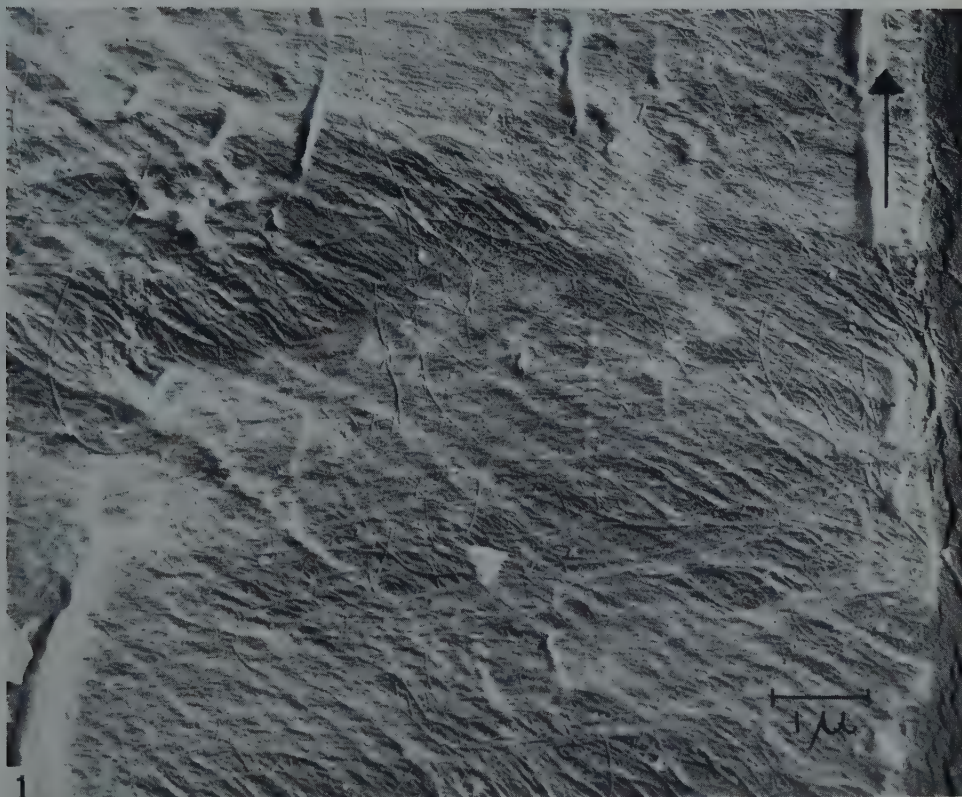
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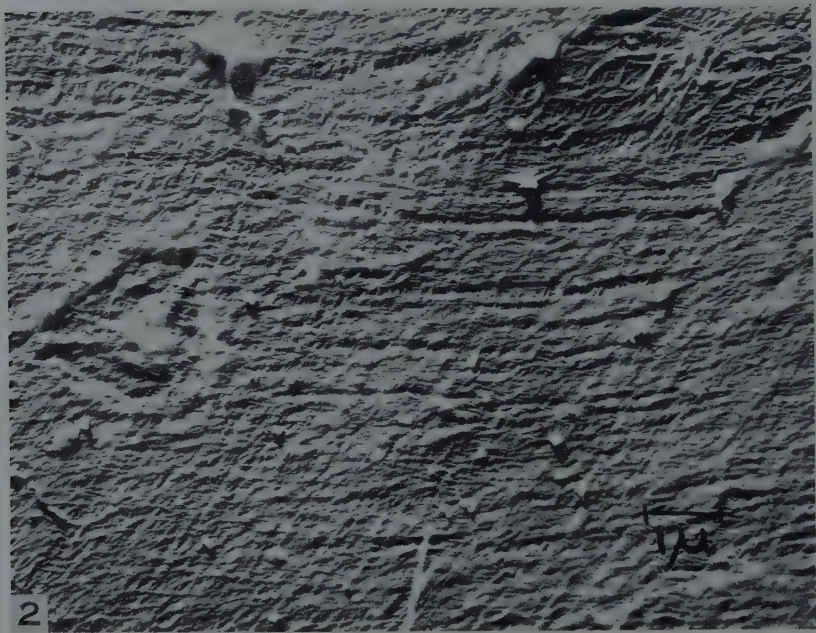
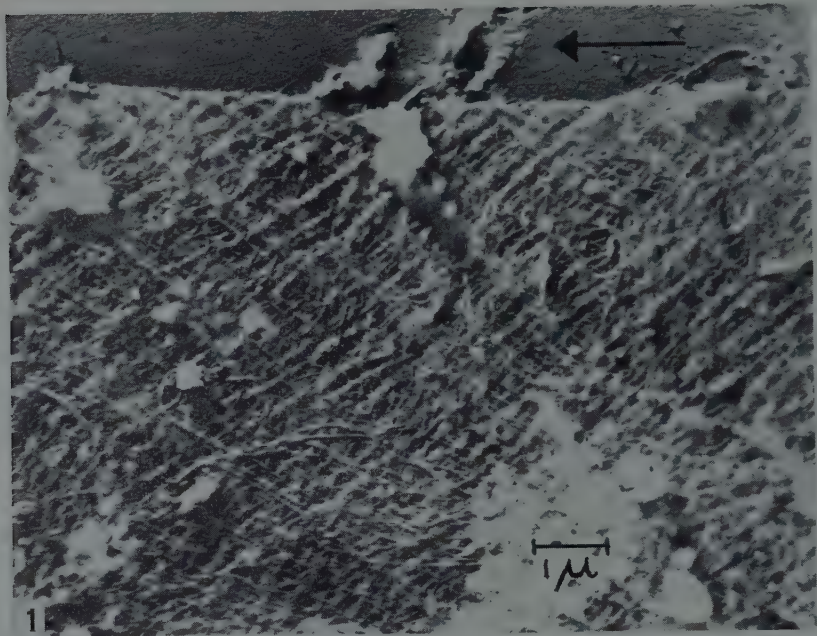
SURFACE GROWTH IN PARENCHYMA OF OAT COLEOPTILES



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EXPLANATION OF PLATES 1-6

All electron micrographs shown were obtained after the specimens had been shadow-cast with uranium. Arrows indicate the major morphological axis.

PLATE 1

Fig. 1.—Electron micrograph of the inner surface of a coleoptile (1.5 cm) parenchyma cell showing regular approximately transverse orientation of microfibrils. $\times 9000$.

Fig. 2.—Electron micrograph showing the outer (O) and inner (I) surfaces of a coleoptile (1.5 cm) after extension at 2–4°C. Note the disorientation of microfibrils on both the inner and outer surfaces. $\times 18,000$.

PLATE 2

Figs. 1 and 2.—Electron micrograph of the outer surfaces of coleoptile parenchyma cells (2–4 mm). Note the approximately longitudinal orientation of microfibrils adjacent to the pit fields. Fig. 1, $\times 11,000$; Fig. 2, $\times 15,000$.

PLATE 3

Fig. 1.—Electron micrograph of the outer surface of a coleoptile (2 cm) parenchyma cell after extension at 2–4°C, showing a band of longitudinally oriented microfibrils. $\times 11,000$.

Fig. 2.—As Figure 1, showing a pit field covered with longitudinally arranged microfibrils. $\times 12,000$.

PLATE 4

Fig. 1.—Electron micrograph of the outer surface of a coleoptile (1 cm) parenchyma cell, showing the disorientation of microfibrils around pit fields and the more perfect alignment of the microfibrils in the longitudinal direction adjacent the pit fields. $\times 15,000$.

Fig. 2.—Parenchyma cell from a coleoptile (1 cm) stained in congo red and photographed in plane-polarized light to show the longitudinal corner thickening. $\times 400$.

Figs. 3 and 4.—Coleoptile (5 cm) parenchyma stained with congo red and photographed between crossed nicols showing the helical secondary cell wall. $\times 400$.

PLATE 5

Fig. 1.—Electron micrograph of a coleoptile (3 cm) parenchyma cell showing what is possibly an early stage in the development of a secondary thickening. $\times 12,000$.

Figs. 2 and 3.—X-ray diffraction diagrams of composite specimens of coleoptiles 1.5 and 5 cm long respectively. Specimen film distance: 3 cm; Cu K_{α} radiation.

PLATE 6

Fig. 1.—Electron micrograph of part of a coleoptile (5 cm) parenchyma cell showing secondary thickening. $\times 9000$. (cf. Plate 4, Figs. 3 and 4.)

Fig. 2.—As Figure 1, but here the specimen appears to be a single wall with crossed fibrillar organization. $\times 9000$.

SOME ANATOMICAL FACTORS RELATING TO THE PENETRATION OF WATER INTO XYLEM OF GYMNOSPERMS

By A. B. WARDROP* and G. W. DAVIES*

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Summary

A study has been made of some sapwood specimens of *Pinus radiata* D. Don and *Pinus muricata* D. Don known to resist penetration by aqueous preservatives but readily penetrated by creosote oil. A few pits only in these specimens showed aspiration. An arbitrary sinkage time has been used as an indication of the penetrability of the wood and it has been shown that pre-extraction with alcohol and ether greatly reduces the sinkage time in water. In *Pinus radiata* and *Picea* spp. the sinkage time was greatly increased by drying but this increase did not take place if the specimens were pre-extracted with alcohol and ether. Extraction after drying at 102°C did not reduce the sinkage time.

A study of staining reactions of the wood with sudan black and sudan IV indicated the presence of fatty material, removable by successive extractions with alcohol and with ether, lining the lumen of pit chambers of the tracheids. This fatty material appeared to be part of a thicker membrane which stained strongly with osmic acid, and could be removed only by extraction with urea. Isolation of this osmiophilic membrane and subsequent electron microscopic examination showed it to have a structure similar to the membrane with "wart-like" texture described by other workers.

I. INTRODUCTION

The influence of bordered pits in governing the penetration of liquids into wood is widely appreciated in the literature of wood technology. This stems from the fundamental studies of pit anatomy by early botanists and the demonstration by Bailey (1913), Frenzel (1929), and subsequently by Liese (1956) that the pit membrane contains perforations permitting the passage of liquids or fine suspensions through them. It was shown further (Bailey 1913; Griffin 1919, 1924) that aspiration of the pit membranes and consequent closing of the pit aperture by the torus greatly reduced the penetration of the wood by liquids.

Another structural feature thought to influence penetration of liquids into wood is the existence of a "non-cellulosic" membrane lining the lumina of the cells. Thus, it was shown initially by Kobayashi and Itsumi (see Harada 1953) and subsequently from the work of Harada and Miyazaki (1952) and Liese and Fahrenbroch (1952) that lining the lumen of the tracheids there is a thin membrane of wart-like texture adpressed to the lumen surface of the secondary cell wall. This membrane also extends into the pit chamber. The membrane is of variable texture and has been recorded in a large number of species, but is not present in all species which have been studied. The excrescences ("warts") on this membrane measure 0.04–0.28 μ in diameter (Harada 1955). It was suggested by Liese and Hartmann-Fahrenbroch (1953) that when a membrane with wart-like texture is present the torus cannot completely close the pit aperture, and so species containing this membrane are more easily penetrable

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by liquids than are those in which it is absent or poorly developed. This membrane was assumed by Wardrop and Dadswell (1957) to represent at least in part the residue of the denatured cytoplasm.

Other factors have also been shown to affect penetration. Thus, Sandermann and Jonas (1952) demonstrated that pre-extraction of wood with methanol facilitated subsequent penetration and it had been shown by Phillips (1933) that drying wood after ethanol extraction resulted in decreased aspiration of the pits, presumably because of the lower surface tension of the ethanol.

The study described in this paper arose from the observation of Mr. F. Dale of the Wood Preservation Section of this Division that certain specimens of *Pinus radiata* and *Pinus muricata* were penetrated readily by creosote oil but not by aqueous preservatives. In these specimens, few pits were aspirated. In view of the work by Sandermann and Jonas (1952) this suggested that there may be some hydrophobic material present which acts as a barrier to penetration by aqueous preservatives. It thus appeared of some importance to investigate the problem further and to determine the location and nature of the substances impeding penetration. Accordingly the following preliminary study has been made.

II. EXPERIMENTAL AND RESULTS

(a) *The Effect of Pretreatments of Wood on Penetration by Water*

As an index of the penetrability of wood by water the sinkage time determination essentially along the lines used by Stone (1956) was employed. Blocks of wood ($\frac{1}{2}$ -in. cubes) were placed in a flask and evacuated for 30 min. Water was then admitted while the specimens were still under vacuum and the time taken for them to sink was noted. Five blocks were used for each measurement. The defects of such a method are appreciated, but it was considered adequate for the present series of observations. Initially, experiments were carried out on specimens of *Pinus radiata* D. Don and *Pinus muricata* D. Don from South Australia, which had been air-dried for an unknown period. In these specimens the sinkage time of sapwood was determined both before and after oven-drying at 102°. It was found that in all cases the sinkage time was greater than 72 hours, but in the oven-dried specimens the sinkage time was greater than this and as high as 14 days. Since these specimens could be penetrated by creosote but not by aqueous preservatives, similar specimens were extracted in a Soxhlet extractor for two hours with ethanol followed by 2 hours extraction with ether. For the oven-dry specimens the sinkage time remained greater than 72 hours, but for the untreated specimens it was reduced to less than 15 sec. Extraction of the heartwood was without effect.

Following these initial observations work was continued on a specimen of *Pinus radiata* from Mount Macedon, Vic., and the observations recorded in Table 1 were made on the sapwood of the outermost three growth rings. From the results of treatment 2 (Table 1) it can be seen that drying for 4 hours at 102°C increased the sinkage time, as was also observed with the samples from South Australia. From treatment 5 it can be seen that this effect of temperature on sinkage time is nullified by pre-extraction with alcohol and ether. Furthermore from treatments 1, 2, 3, and 4 (Table 1) it is apparent that both the period and temperature of drying governed the

extent of the increase in sinkage time which occurred. It appears from the results that drying in the field may well have been responsible for the initially high sinkage time and consequent low penetrability observed in the specimens collected in South Australia.

These results suggested the presence in the wood of an alcohol—ether soluble fraction which hindered the penetration of the wood by water and which, after prolonged drying, was fixed either by drying, or oxidation, or both so that it could no longer be extracted by these solvents (treatment 7, Table 1). An alternative possibility was that drying caused severe aspiration of the pits and that when newly collected wood was extracted by alcohol and ether, aspiration of the pits did not occur. This possibility was suggested by the observation of Phillips (1933) that drying wood from alcohol rather than from water greatly reduced pit aspiration. However, from treatment 6 (Table 1), it can be seen that if after extraction with alcohol and ether the

TABLE 1

EFFECT OF VARIOUS PRETREATMENTS ON THE SINKAGE TIME OF SAPWOOD FROM *PINUS RADIATA**
(MACEDON, VIC.)

| Treatment No. | Treatment | Sinkage Time (hr) |
|---------------|--|-------------------|
| 1 | Nil | 0 |
| 2† | Dried in air oven at 102°C— 0.5 hr | 0 |
| | 1.0 hr | 0 |
| | 2.0 hr | 0 |
| | 4.0 hr | >72 |
| 3 | Dried to constant weight over phosphorus pentoxide, room temperature | 5 |
| 4 | Dried to constant weight at 40°C | >72 |
| 5 | Extraction 2 hr alcohol, 2 hr ether, dried 4 hr at 102°C | 0 |
| 6 | As for 5, with additional soaking in alcohol and water before drying | 0 |
| 7 | Dried 4 hr at 102°C. Extraction 2 hr alcohol, 2 hr ether | >72 |

*Similar results were obtained with *Picea* sp.

†The period of drying after which the large increase in sinkage time occurs varies greatly with wood from different trees.

specimens were soaked in water before drying the reduction in sinkage time was still observed. Thus it was concluded that pit aspiration was not in this case the cause of the effects observed and an attempt was made, therefore, to determine the location of the alcohol—ether soluble fraction of the wood. Similar observations to those recorded in Table 1 were made on a specimen of *Picea* from Macedon, Victoria.

(b) Anatomical Observations

(i) *Staining Reactions with Sudan IV and Sudan Black.*—With sudan IV and sudan black similar general staining reactions were observed in *Pinus radiata* (South Australia and Macedon), *Pinus muricata* (South Australia) and *Picea* sp. (Macedon). With sudan black the untreated material of *Pinus radiata* (Macedon) showed heavy staining in the ray parenchyma and particularly the ray tracheids, and in the pit

chambers of the tracheids and of the ray tracheids, and less intense staining around the edge of the lumen. On extraction before staining virtually all the stainable material was removed.

With the dried material of all specimens similar staining reactions were observed (Plate 1, Fig. 1), but after extraction some staining was still observed in the rays and pit chambers (Plate 1, Fig. 2). With sudan IV, the staining reaction in the pit chamber and lumina of the tracheids was much less intense than with sudan black.

However, in fresh material (Macedon), fat droplets observed in the ray parenchyma (Plate 1, Fig. 3) appeared to coalesce on drying and formed large aggregates spreading over the inner surface of the cell wall as drying progressed (Plate 1, Fig. 4). Extraction before drying completely removed this material.

If it is assumed that the substances staining with sudan IV are fats, and those staining with sudan black consist of fats or lipoids, it is clear that these observations are consistent with the idea that the increased difficulty of penetration of the wood by water after drying is related to the presence of these substances, particularly in the pit chambers. The extract stained strongly with sudan black and sudan IV and had an oily appearance.

(ii) *Staining Reactions with Osmic Acid*.—Both untreated and dried blocks of all specimens were stained with 2 per cent. osmic acid for 24 hours. After washing and sectioning, a well-defined membranous structure could be recognized lining the lumen and the pit chambers. This osmiophilic membrane was better defined in the dry sections than in the fresh sections. It can be seen lining tracheid lumina and pit chambers in Plate 2, Figures 1 and 2, and the ray tracheids of *P. radiata* in Plate 2, Figure 3. For the most part this membrane lies inside the birefringent inner layer of the secondary wall in *P. radiata*, as was shown by examination between crossed nicols (Plate 3, Figs. 1 and 2).

The usual alcohol extraction followed by ether extraction failed to remove this membrane in both fresh and dry material, so this membrane did not appear to influence the ease of penetration of the wood by water. Neither could it be removed by extraction with chloroform or acetone, but prolonged extraction in urea (5.0M) did remove it from the cell lumina but not from the pit chambers. In urea-extracted sections staining was observed to be very intense in the pit chambers and the radial strands of the pit membrane (Plate 3, Fig. 3).

From these observations it is clear that in addition to the fatty material detected by the sudan stains, there exists a resistant membrane lining the lumen and pit chambers. To examine this membrane further the question arose whether it could be isolated. This was found to be possible by cutting longitudinal sections 20 μ thick from the osmium-stained blocks and treating them in a mechanical disintegrator. By this means fragments of the osmiophilic membrane could be recognized because of their dark colour among the cell debris. The membrane was isotropic when viewed between crossed nicols and thus could be assumed not to contain cellulose (Plate 3, Figs. 4 and 5).

The supernatant of the suspension of disintegrated sections was then examined in the electron microscope after uranium shadowing. Such fragments are shown in Plate 4, Figures 1 and 2, and Plate 5, Figure 1.

(iii) *Staining Reactions for Protein*.—Staining reactions for protein gave positive results only with the potassium ferrocyanide–ferric chloride reaction described by Gomori (1952). Isolated patches of blue membranous material were observed in the tracheid lumina and in the pit chambers (Plate 3, Fig. 6). This staining lacked the regularity of the staining with osmic acid. However, its similar location suggested that at least the material stained by the protein reagent was associated with the osmiophilic membrane. This stained material could be isolated from the supernatant of a suspension in water of the mechanically disintegrated sections. It was then examined in the electron microscope after uranium shadowing. For the most part this material did not show the wart-like structure of the osmiophilic membrane (Plate 5, Fig. 2) but thicker fragments did have some granular structure (Plate 5, Fig. 3).

III. DISCUSSION

From the above study of the sinkage time of small wood specimens, it appears that there exists an alcohol–ether soluble fraction of the wood which, after drying of the specimen, hinders the penetration of water into wood. This view is supported by the observation that pre-extraction of wood before drying greatly reduces the observed sinkage time of the specimens. From the observation (Table 1) that extraction after drying does not similarly reduce the sinkage time, it appears that the alcohol–ether soluble fraction becomes fixed in the wood, either as a result of drying or drying coupled with oxidation. The reduction in staining reactions with sudan IV (fat) and sudan black (fat or lipid) as a result of extraction suggests that this material is at least partly fatty in nature. In relation to its fixation on drying, it is perhaps significant that as cut wood ages the alcohol–ether soluble fraction progressively decreases (Schwalbe and Schulz 1918). The existence of such a fatty layer, lining the lumina of cells after death, is also understandable in view of the recorded fat and lipid content of the cytoplasm of 2–20 per cent. (see, for example, Stiles 1950) and of the values of 0.75–2.00 per cent. recorded by Arrhenius (1942) for the fat content of wood in aspen, birch, and spruce. Furthermore, the occurrence of fatty substances in the cell lumina and pit chambers is consistent with the observations of Scott (1955) in living cells that fat droplets may frequently be observed in the parietal cytoplasm and plasmodesmata. Because of their location it is possible that these fatty substances are associated with, or included in, the membrane stained with osmic acid (Plate 2), but since the bulk of this membrane could not be extracted with alcohol and ether it is clear that only the fatty substances staining with the sudan stains affected the ease of penetration of water into the wood. A comparison of sections stained by sudan black and by osmic acid showed that substances other than fats are present in the osmiophilic membranes. That these substances may be in part protein is suggested by evidence such as that in Plate 3, Figure 6, and the lack of birefringence in this membrane would indicate the absence of cellulose (Plate 3, Figs. 4 and 5).

Electron micrographs of the osmiophilic membrane (Plate 4) suggest that this corresponds to the membrane with wart-like structure described originally by Kobayashi and Itsumi (see Harada 1953) and later by Liese and Fahrenbroch (1952) and by Harada (1953). The “warts” on this structure fall within the dimensional range of 0.04–0.28 μ given by Harada (1955). From an examination of Plate 4, it can be

seen that the "warts" appear to be embedded in, or supported by, an amorphous membrane which shows superficial resemblance to the layer stained by protein reagent shown in Plate 5, Figure 2, and also to fat globules seen in the supernatant of the homogenized osmium-stained sections (Plate 5, Fig. 4). From the shadow length this membrane was calculated as approximately 0.05μ in thickness. It may be noted, too, that in the photomicrograph of the osmiophilic layer in Plate 2, Figure 2, small dark spots can be seen which may correspond to the "warts" in the electron-micrographs of Plate 4.

Because the osmiophilic membrane gives negative reactions for cellulose, and because of its location, demonstrated in Plate 3, Figures 1 and 2, it is obvious that it is in no sense part of the cell wall. Furthermore, since it is accentuated in dead cells (after drying) it is reasonable to regard it as a cytoplasmic residue representing the remnants of the disorganized protoplasts. From observations made so far this membrane staining with osmic acid is recognizable in widely separated genera (*Pinus*, *Picea*, *Eucalyptus*). It is not clear, however, whether the membrane has the "wart-like" structure in all instances. Thus, Frey-Wyssling, Muhlethaler, and Bosshard (1956) report in a survey of gymnosperms that the "wart" structure appears to be of diagnostic significance, being found only in those genera with dentate ray tracheids. These observations would suggest either that the "warts" represent some special structures produced by the cytoplasm, or if they represent a cytoplasmic residue, as suggested previously (Wardrop and Dadswell 1957), then the cytoplasm must have characteristic inclusions in different genera. Consideration of this question must await further investigation.

From the present study, however, it is clear that the osmiophilic layer with wart-like texture does not itself impede penetration of water into the wood, but penetration is impeded by the fatty material associated with it. Although the results here presented are obviously of limited scope and have yet to be correlated with practical treatments for preservation of wood, they perhaps draw attention to the fact that drying (aging) of wood may be of significance in affecting the ease of penetration by aqueous preservatives, and that excessive drying may produce undesirable changes.

IV. REFERENCES

- ARRHENIUS, O. (1942).—*Svensk. bot. Tidskr.* **36**: 95.
 BAILEY, I. W. (1913).—*For. Quart.* **11**: 12.
 FRENZEL, P. (1929).—*Planta* **8**: 665.
 FREY-WYSSLING, A., MUHLETHALER, K., and BOSSHARD, H. H. (1956).—*Holz a. Roh-u. Werkst.* **14**: 162.
 GOMORI, G. (1952).—"Microscopic Histochemistry." (University of Chicago Press.)
 GRIFFIN, G. J. (1919).—*J. For.* **17**: 813.
 GRIFFIN, G. J. (1924).—*J. For.* **22**: 82.
 HARADA, H. (1953).—*J. Jap. For. Soc.* **35**: 393.
 HARADA, H. (1955).—*J. Jap. Wood. Res. Soc.* **1** (2): 85.
 HARADA, H. and MIYAZAKI, Y. (1952).—*J. Jap. For. Soc.* **34**: 350.
 LIESE, W. (1956).—Proc. Int. Conf. on Electron Microscopy (Stockholm), p. 550.
 LIESE, W., and FAHNENBROCH, M. (1952).—*Holz a. Roh-u. Werkst.* **10**: 197.
 LIESE, W., and HARTMANN-FAHNENBROCH, M. (1953).—*Biochim. Biophys. Acta* **11**: 190.
 PHILLIPS, E. W. J. (1933).—*Forestry* **7**: 109.
 SANDERMANN, W., and JONAS, G. Z. (1952).—*Holz. a. Roh-u. Werkst.* **10**: 305.

SCHWALBE, C. G., and SCHULZ, W. (1918).—*Z. angew. Chem.* **31**: 125.

SCOTT, F. M. (1955).—*Amer. J. Bot.* **42**: 475.

STILES, W. (1950).—"Principles of Plant Physiology." 2nd Ed. (Methuen & Co.: London.)

STONE, J. E. (1956).—Canada Pulp and Paper Assn. Tech. Sect. Proc., p. 347.

WARDROP, A. B., and DADSWELL, H. E. (1957).—*Holzforschung* **11**: 33.

EXPLANATION OF PLATES 1-5

PLATE 1

Fig. 1.—*Pinus radiata* (South Australia): Tangential longitudinal section of air-dry wood stained with sudan black. $\times 100$.

Fig. 2.—as Figure 1, after extraction for 2 hours with alcohol followed by 2 hours with ether. $\times 100$.

Fig. 3.—*Pinus radiata* (Macedon): Radial longitudinal section of fresh wood stained with sudan IV. Note the fat globules. $\times 430$.

Fig. 4.—as Figure 3, after oven-drying. Note that the fat globules have coalesced and tend to spread over the inner surface of the ray parenchyma. $\times 430$.

PLATE 2

Fig. 1.—*Pinus radiata* (South Australia): Radial longitudinal section of air-dry wood stained 24 hours with 2 per cent. osmic acid. Note the membranous appearance of the osmium-stained material. $\times 430$.

Fig. 2.—as Figure 1, showing intense staining in the pit chambers. $\times 1000$.

Fig. 3.—as Figure 2. Note the heavy staining in the ray tracheids, but not parenchyma. $\times 430$.

PLATE 3

Fig. 1.—*Pinus radiata* (South Australia): Transverse section of air-dry wood stained 48 hours in 2 per cent. osmic acid. $\times 1000$.

Fig. 2.—The same section as shown in Figure 1 viewed between crossed nicols. Note that the osmiophilic layer appears to be inside the inner birefringent layer of the secondary wall. $\times 1000$.

Fig. 3.—A radial longitudinal section of air-dry *Pinus radiata* (South Australia) extracted with 5.0M urea showing the staining with osmic acid of the pit membrane. $\times 2500$.

Fig. 4.—A fragment of cell wall, *W*, with an attached part of the osmiophilic membrane, *O*, isolated by mechanical disintegration of sections cut from a block of *Pinus radiata* (air-dry, South Australia) stained with osmic acid. $\times 430$.

Fig. 5.—As Figure 4, viewed between crossed nicols. $\times 430$.

Fig. 6.—*Pinus radiata* (air-dry, South Australia): Radial longitudinal section stained using the Hartig-Zacharias reaction for protein. $\times 430$.

PLATE 4

Figs. 1 and 2.—Isolated fragments from sections of *Pinus radiata* (South Australia) showing material stained with osmic acid, viewed in the electron microscope. Uranium shadowed. Note the wart-like texture. cf. Plate 2, Figures 1 and 2. $\times 18,000$.

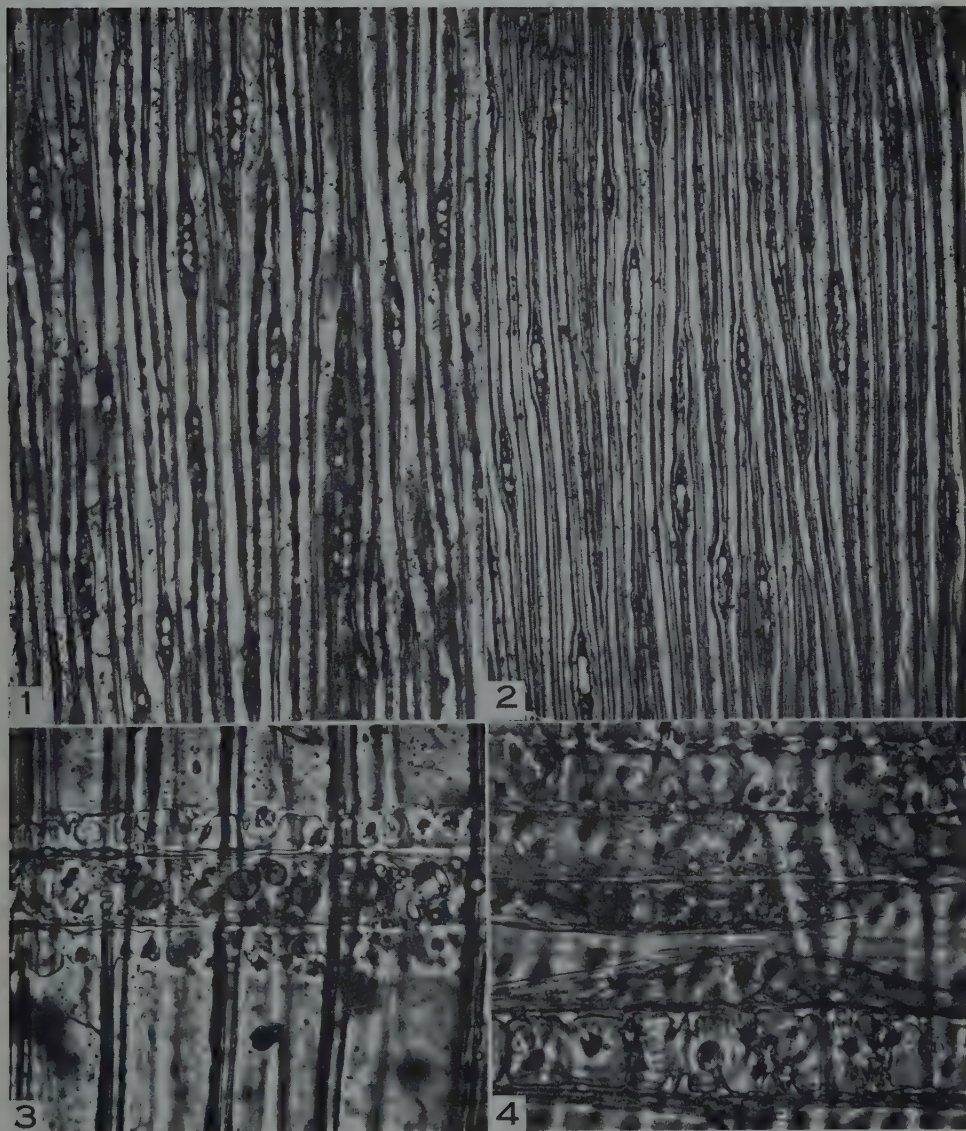
PLATE 5

Fig. 1.—*Pinus radiata* (South Australia): An electron micrograph of a thick fragment of cell wall overlain by the osmiophilic membrane. Uranium shadowed. $\times 11,250$.

Figs. 2 and 3.—*Pinus radiata* (South Australia): Electron micrograph of isolated fragments of material staining for protein. Uranium shadowed. cf. Plate 3, Figure 6. $\times 13,000$.

Fig. 4.—Electron micrograph of debris observed after mechanical disintegration of section of *Pinus radiata* (South Australia) stained with osmic acid. Uranium shadowed. cf. the non-wart-like material of Plate 4. $\times 13,000$.

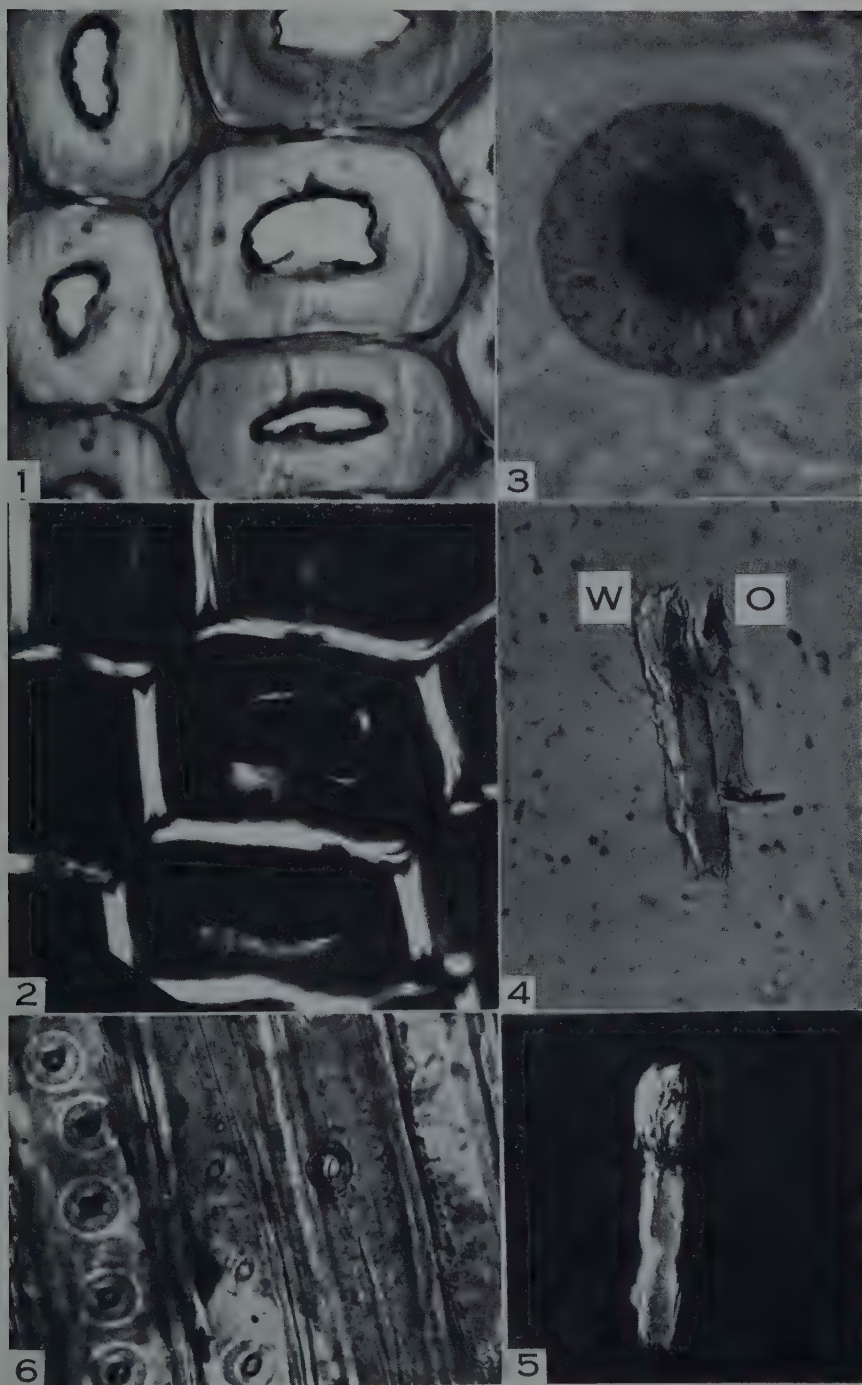
PENETRATION OF WATER INTO XYLEM OF GYMNOSPERMS



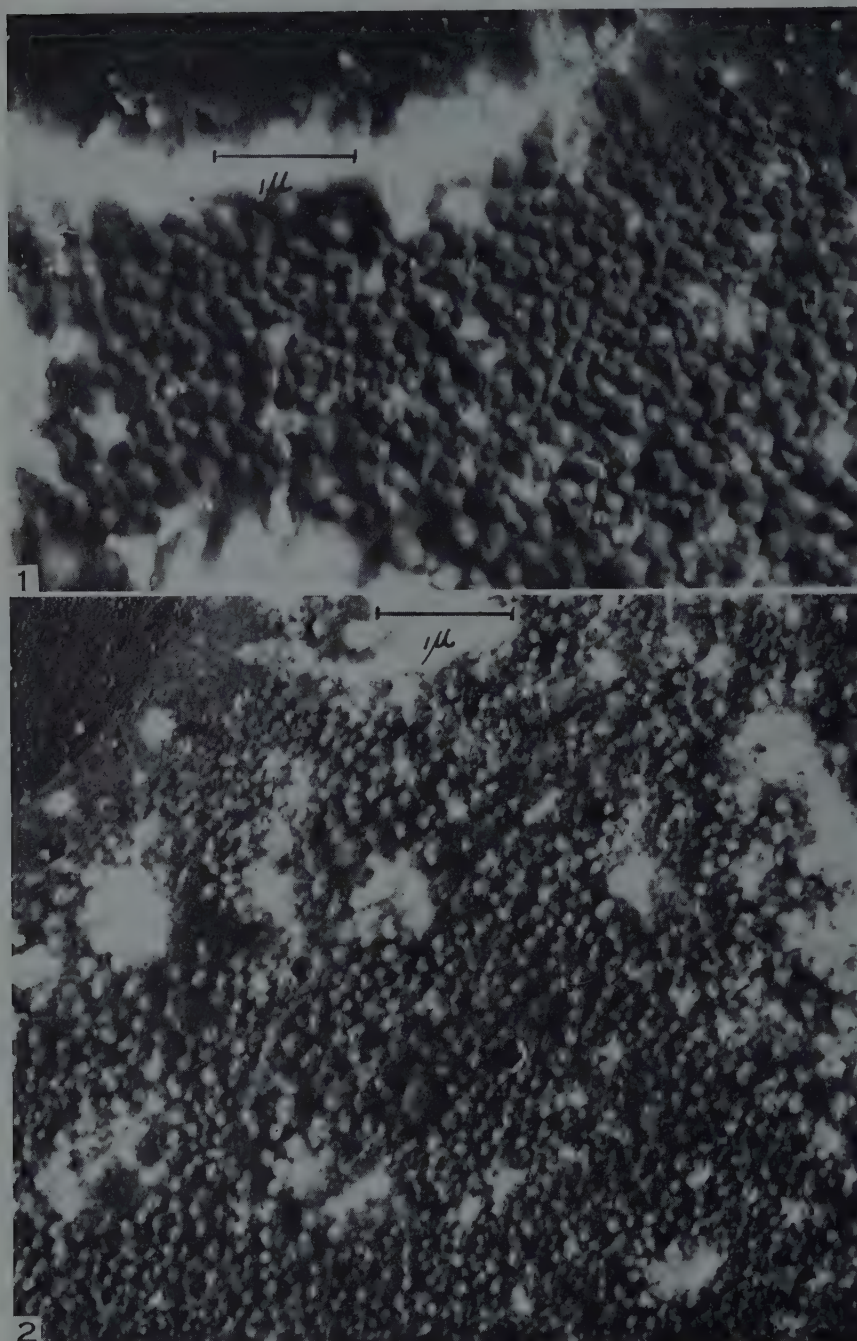
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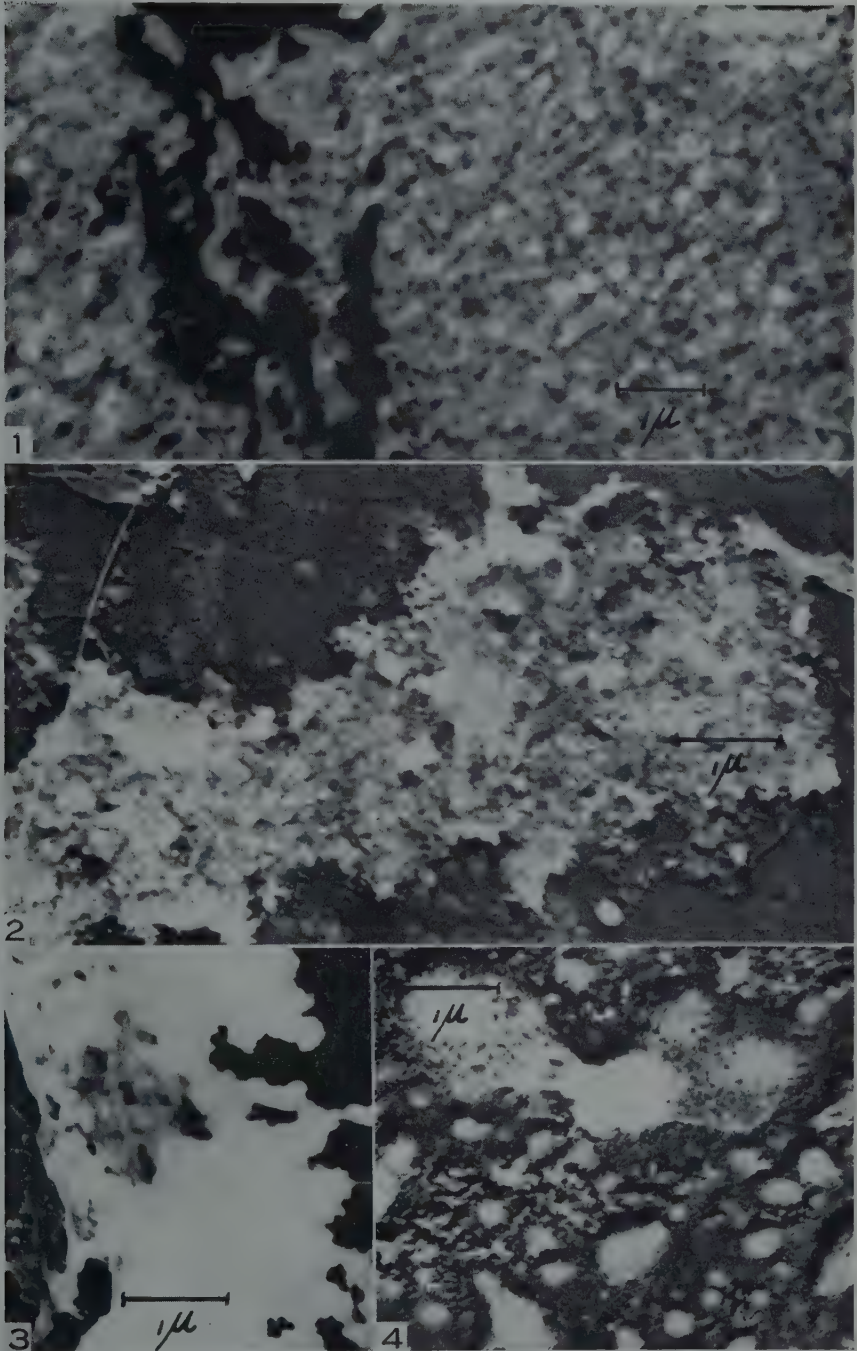
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PENETRATION OF WATER INTO XYLEM OF GYMNOSPERMS



PENETRATION OF WATER INTO XYLEM OF GYMNOSPERMS



BUD DEVELOPMENT AND LIGNOTUBER FORMATION IN EUCALYPTS

By M. MARGARET CHATTAWAY*

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Summary

The minute structure of lignotubers is described and their development studied from their earliest inception in the cotyledonary and lower leaf nodes.

The structure of the bud components of the leaf and cotyledonary nodes of tubering and non-tubering species has been compared. Lignotubers can only develop in species in which concealed buds are present above as well as below the axillary buds. Other factors may, however, inhibit their development even when the necessary buds are present.

I. INTRODUCTION

The great regenerative capacity of many eucalypt species after a forest fire and the rapidity of their height growth at the sapling stage are among the first peculiarities of these trees to catch the attention of overseas visitors, especially those used to the deciduous forests of the northern hemisphere.

The part played by the concealed bud in bringing about the former and that of the naked bud in contributing to the latter have been discussed by Jacobs (1936, 1955). He, however, dealt with these problems from the silvicultural rather than the anatomical angle and his comments on the differences between the different buds are based on macroscopic examination only.

The work reported here is a study of the anatomical structure of the different bud types found on lignotubers in leaf nodes and in the cotyledonary nodes of the eucalypts. It is an attempt to explain how it is that, whereas lignotubers are a constant feature of the seedling and sapling stages of many eucalypts, there are some species in which they have so far never been observed. As the lignotubers arise in the axils of the cotyledons and in the first few leaf axils, these have been studied from the cotyledonary stage up to the development of a definite stem swelling.

The problem is important from a practical angle, as some of the most useful timber trees are among those which do not have lignotubers.

II. MATERIAL AND METHODS

Both the leaf nodes and the cotyledonary nodes of seedlings were cut into serial sections on a microtome after embedding in methacrylate. The following species have been examined.

Leaf nodes: *Eucalyptus eximia* Schau., *E. goniocalyx* F. Muell., *E. hemiphloia* F. Muell., *E. radiata* Sieb.

Cotyledonary nodes: *E. bicolor* A. Cunn., *E. eremophila* (Diels) Maiden; *E. linearis* Dehn., *E. macrandra* F. Muell., *E. oleosa* F. Muell., *E. pulverulenta* F. Muell., *E. punctata* DC.

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Cotyledonary and leaf nodes: *E. astringens* Maiden, *E. bicostata* Maiden, Blakely & Simmonds, *E. botryoides* Sm., *E. calycogona* Turcz., *E. camaldulensis* Dehn., *E. citriodora* Hook., *E. cladocalyx* F. Muell., *E. diversicolor* F. Muell., *E. fastigata* Deane & Maiden, *E. ficifolia* F. Muell., *E. flocktoniae* Maiden, *E. fraxinoides* Dean & Maiden, *E. gardneri* Maiden, *E. gigantea* Hook.f., *E. globulus* Labill., *E. gomphocephala* DC., *E. gracilis* F. Muell., *E. gummifera* (Gaertn.) Hochr., *E. lehmanni* Preiss, *E. leucoxydon* F. Muell., *E. maculata* Hook., *E. marginata* Sm., *E. melliadora* A. Cunn., *E. microcorys* F. Muell., *E. microtheca* F. Muell., *E. nitens* Maiden, *E. obliqua* L'Hér., *E. occidentalis* Endl., *E. oreades* R. T. Bak., *E. pilularis* Sm., *E. polyanthemos* Schau., *E. redunca* Schau., *E. regnans* F. Muell., *E. resinifera* Sm., *E. rudis* Endl., *E. scabra* Dum-Cours, *E. sideroxydon* A. Cunn., *E. sieberiana* F. Muell., *E. smithii* R. T. Bak., *E. viminalis* Labill.

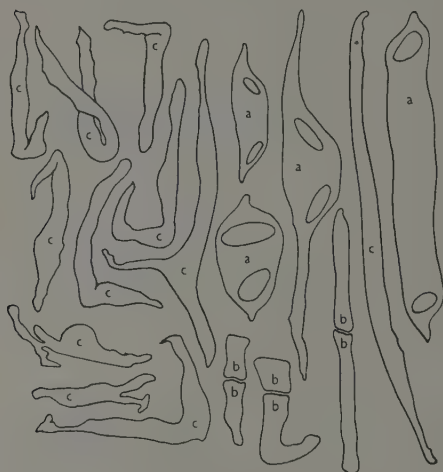


Fig. 1

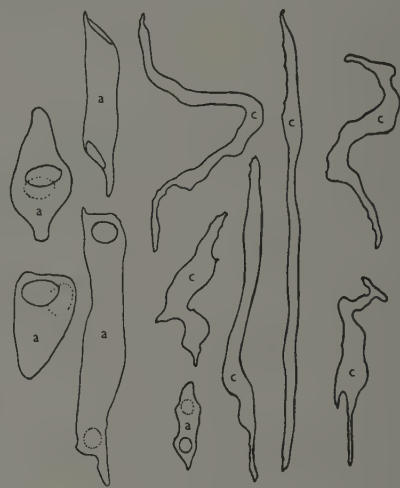


Fig. 2

Fig. 1.—*E. obliqua*. Elements of lignotuber as seen in macerated material. *a*, Vessel members; *b*, ray or parenchyma cells; *c*, fibres or tracheids. $\times 200$.

Fig. 2.—*E. maculata*. Elements of lignotuber as seen in macerated material. *a*, Vessel members; *b*, ray or parenchyma cells; *c*, fibres or tracheids. $\times 200$.

III. LIGNOTUBERS

Our knowledge of the structure of lignotubers rests mainly on the work of Kerr (1925) and Carter (1929), who described lignotubers anatomically and listed species in which they were known to occur.

It is unfortunate that Kerr did not recognize that the woody elements of the lignotuber are fundamentally similar to those of the stems on which they occur. She coined the word "phlaeotracheae", which she used for all the elements of the lignotuber except the ray cells. The pitted elements to which she applied this term are, in fact, the tracheids and fibre-tracheids of the normal eucalypt stem, much distorted and twisted, and performing the function of storage tissue. Among them

there are also vessels and parenchyma cells, which are not mentioned by Kerr. Figures 1 and 2, of macerated lignotubers of *E. obliqua* and *E. maculata*, show some of the elements in outline.

It would be a good thing if the term phlaeotracheae were allowed to lapse. Plate 1, Figure 1, of a young lignotuber of *E. viminalis* shows how the normal (*a*) and distorted (*b*) wood merge into one another, so that the use of a separate term to describe the lignotuber tissue is difficult to apply and confusing. Owing to the disintegration inherent in macerations the rays and parenchyma strands fall apart and it is difficult to tell the cells of the one from those of the other. Nevertheless, all the elements of the normal stem can be recognized in sections of lignotubers, although the picture is often confused. The huge distortion undergone by the tracheids and fibre-tracheids makes these elements difficult to distinguish from one another both in sections and in macerations of lignotubers. In normal wood the former are more heavily pitted; in wood from lignotubers, differences in pitting can be seen in the twisted elements and may be used as an indication of the true nature of the several cells.

The greatest tissue distortion usually seems to occur near the vascular strands by which the buds are connected with the central normal portion of the lignotuber (Plate 1, Fig. 4), although the strands themselves are not the centre of the tissue "swirl". Jacobs (1955) has described the way in which the strands to the concealed buds persist as the tree increases in girth, multiplying to produce a series of buds in the older tree. Similar development in the lignotuber produces a series of concealed buds which can be recognized as small points on the surface of the lignotuber. These buds may occur in either horizontal or vertical series, or may be in irregular clusters.

Both Kerr and Carter were mistaken in thinking that the endogenous bud ate its way out to the surface by means of a digestive cap. The tissue shown in Kerr's figure (Kerr 1925, Fig. 3, p. 88) is in fact leaf tissue. The leaves of these, and of the concealed buds of the stem, overarch the apex in a decussate arrangement. On sectioning, part of a leaf is often cut through, and this can give the impression of a cone of tissue overarched the growing point. Serial sections, however, show that it is part of a leaf lying behind or in front of the growing point. Plate 1, Figures 2 and 3, show an endogenous bud from a lignotuber of *E. melliodora*. Plate 1, Figure 2 shows the position of the bud in relation to the strands from previously removed shoots, and Plate 1, Figure 3 shows the details of its structure and of the tissue outside it at a higher magnification. This bud is at an early stage of development and the young leaves have not yet separated from the mass of undifferentiated meristematic cells. There is no digestive cap.

Kerr has demonstrated the endogeny of the buds on the lignotuber. They arise in the cortex of very young lignotubers and in the phloem of older ones. In structure they are similar to the concealed buds of the leaf axils, having short internodes and incurving, overarched semi-protective leaves. These open into small rounded leaves, often red in colour. As soon as the emerging shoot has produced two or three of these leaves the structure of the bud passes into that of a naked bud, from which the juvenile leaves normal to the species are formed. Later these in their turn give way to the mature leaf form.

The importance of the lignotuber to the young eucalypt lies in its regenerative power, in the food reserves it contains, and in the dormant buds from which new leading shoots can be quickly produced when the need arises. Not all eucalypts have lignotubers, and, although lignotubers develop to a larger size in some of the species that grow in the drier areas of Australia, neither the habitats nor relationships of the species give the clue to their production. For example, *E. regnans* growing in moist forests is without lignotubers, yet they occur in *E. obliqua*, its close relative, which may be growing alongside it, and in *E. goniacalyx*, an unrelated species in the same forest.

The solution of this problem seems to lie, not in the study of the mature lignotuber, but in the nodal structure of the young seedlings in the cotyledonary axils and lower nodes of which they first arise.

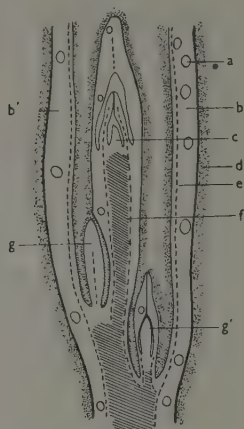


Fig. 3

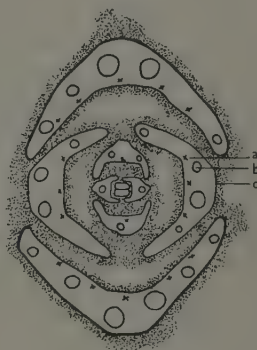


Fig. 4

Fig. 3.—*E. obliqua*. Longitudinal section of naked bud. *a*, Oil gland in leaf; *b*, *b'*, outer expanded leaves; *c*, terminal naked bud; *d*, waxy covering of leaves and buds; *e*, vascular strands; *f*, pith; *g*, *g'*, axillary naked buds. $\times 25$ approx.

Fig. 4.—*E. gummifera*. Transverse section of naked bud. *a*, Vascular strands; *b*, oil glands; *c*, caoutchouc covering of bud. $\times 25$ approx.

IV. NODAL STRUCTURE

(a) General

The leaf nodes of 44 species of *Eucalyptus* have been examined. In spite of differences in detail, the main outlines of their structure follow a definite and regular plan. The apex of the growing shoot consists of a naked bud, of which the detailed structure will be given below. This bud bears pairs of leaves, each of which carries in its axil another naked bud similar in structure to the apical one (Figs. 3 and 4). The naked buds are responsible for the primary growth of the young tree, for height growth, and for the production of the first crop of leaves. In the mature tree they carry on height growth and produce the main crop of leaves for each height storey (Jacobs 1955). They occur in the axil of every leaf, but only a small proportion of

those produced ever reaches maturity; the majority are shed in the first hot dry spell (the line of abscission being similar to that by which the leaves are shed), and their scars become a conspicuous feature of the older leaf axils.

In the axils, between these naked buds and the leaves which subtend them, are patches of meristem from which new buds are produced. These buds, the concealed buds of Jacobs (1936, 1955), have quite a different structure from the naked buds; they function as replacement buds, growing out only after the naked buds or their shoots have dropped off. They provide shoots which will fill gaps in the crown caused by defoliation by insects, drought, etc., and, if they are not used in the early years of the tree's life, the traces that subtend them grow out with the expanding stem, forming a focal point for future epicormic branches. A full account of their macroscopic structure and growth and of their importance in the life of the tree is given by Jacobs (1955).

(b) *The Naked Buds*

Anatomically the chief feature of the naked bud lies in the way in which protection is given to the leaf apex, without the formation of scales, and the provision for rapid extension growth by long internodes.

Protection of the young leaves is simply by covering them with the next older ones in the succession (Fig. 3). There is little of the complicated infolding that is common in the resting buds of deciduous trees, and, as one pair opens and spreads the lamina, its protective function is taken over by the next pair in the succession. In the bloodwood group the young leaves are covered by a tough rubbery substance (Welch 1923); in species from which this caoutchouc is absent the young leaves are heavily cuticularized or are covered with a waxy covering (Fig. 3).

The leaves of the naked buds are straight, the only folding being that of the blade along the midrib (Fig. 4); in very young leaves there may be no folding at all. Naked buds grow very rapidly, the leaves very soon becoming separated by long internodes. Even in the small unexpanded buds the internodes between successive pairs of leaves are quite long, and appear disproportionately long in comparison with those of the scale-covered buds of deciduous trees. Leaf oil glands appear at a very early stage of development, but their number, shape, and distribution vary from species to species.

(c) *The Concealed Buds*

As soon as the axillary buds are injured or cut off, growth of the crown is taken over by the concealed buds. These lie between the naked buds and the petiole and may be either *exogenous* or, less frequently, *endogenous* in origin. In the very early stages of mature nodes they appear as deep-seated, convex, meristematic areas (Plate 1, Figs. 5 and 6), covered by a patch of tanniniferous tissue (Text Fig. 5B). This tissue, to which no reference can be found in literature, may develop on either the axis or the petiole, but is more frequent and of greater size on the petiole. In future it will be referred to as the *protective flap*. It appears to be a special tissue formed at the base of the petiole, or as a small area at the base of the initial axis or the axillary shoot (Plate 1, Fig. 6). The regular rows of cells that produce it can be clearly seen in Text Figure 5B and Plate 1, Figure 5. The outer cells of this area

secrete copious kino—the amount of which varies from species to species—finally disintegrating to form a mass of kino which may almost surround the young bud (Plate 2, Fig. 1). In many eucalypt species the protective flap can easily be seen with the naked eye at the base of most petioles.

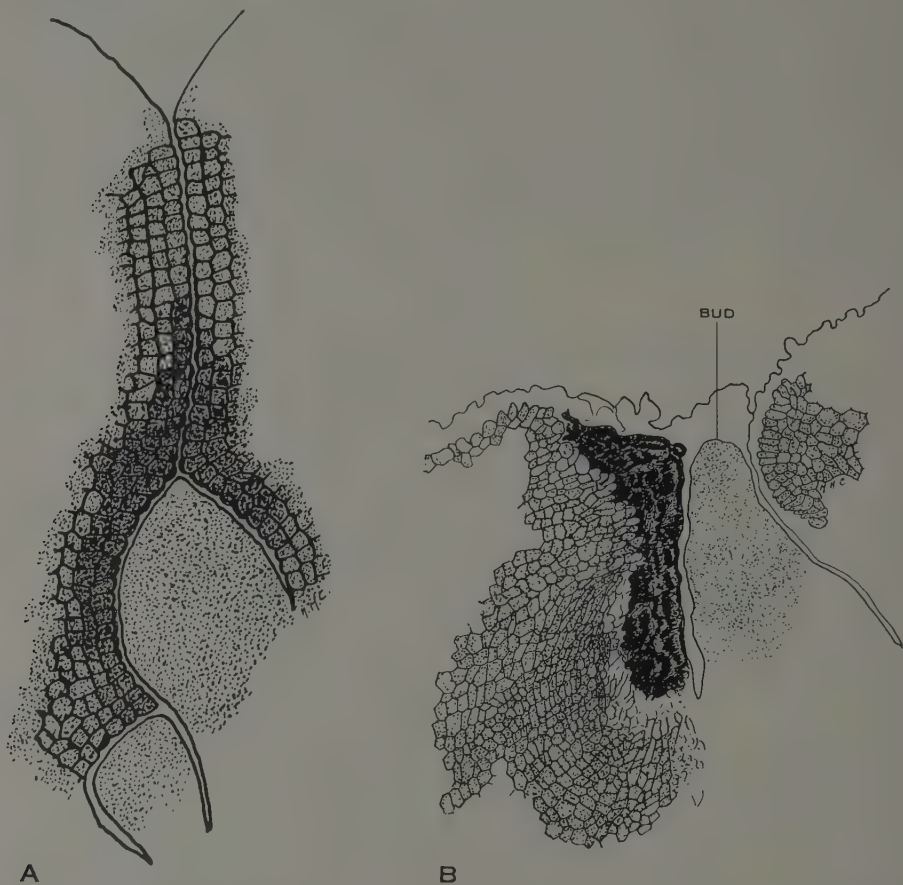


Fig. 5A.—*E. diversicolor*. Young concealed buds, showing close approximation of protective flaps. $\times 200$.

Fig. 5B.—*E. maculata*. Details of protective flap shown in Plate 1, Figure 5. $\times 300$.

From its earliest inception the concealed bud differs in shape from the naked bud. The successive leaf pairs are folded over, giving the bud a rounded appearance. The outer side—the undersurface of the expanded leaf—often has enlarged epidermal cells with heavily cuticularized walls. In the bud the leaf is bent over, which brings these cells into the position of greatest strain as the protective flap is pushed aside. This infolding of the leaf apex contrasts with the straight tips of the leaves of the naked buds, and the leaves that open first on shoots that come from concealed buds differ from those from the naked buds. They have been described by Carey (1931), who terms them *intermediate leaves* or, following the nomenclature used by Foster (1929), *cataphylls*. The cataphylls of all the *Eucalyptus* species examined are rounded and separated by rather short internodes. They are similar in appearance whether

they are produced by a concealed axillary bud at a node or from a proventitious bud on a lignotuber. They appear to have a semiprotective function for the emerging bud, but, after a very few pairs have emerged, the bud reverts to the naked type and the next leaves are the normal juveniles associated with the species, or, in the crown buds, the normal mature leaves of the species.

The meristem of the concealed bud is often very deeply placed at the base of a depression between the petiole and axillary shoot. This is the "invaginated" growing area described by Carey (1931). Sometimes the petiole and the base of the axillary shoot press very closely against one another and give an impression of endogeny (Text Fig. 5A). In the majority of species examined the concealed buds arise exogenously, but very deep-seated buds that are undoubtedly endogenous have been

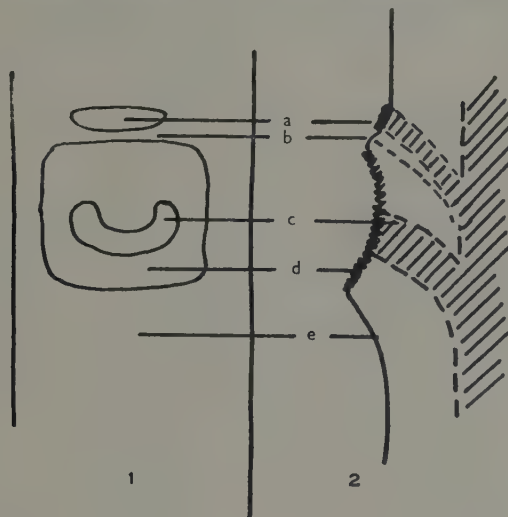


Fig. 6.—Diagram of older node on 2-year old eucalypt stem. 1, surface view; 2, longitudinal section. *a*, Axillary shoot; *b*, meristematic area between shoot and petiole; *c*, strand to petiole; *d*, leaf scar; *e*, surface of stem. $\times 15$ approx.

observed in *E. camaldulensis*, *E. smithii* (Plate 2, Fig. 3), and others. In *E. gracilis*, *E. punctata*, and *E. rudis* (Plate 2, Fig. 4) both exogenous and endogenous buds have been observed. As the endogenous buds increase in size they push their way to the surface, the overlying tissue cracking schizogenously. This crack may continue the line of the depression between petiole and axillary shoot (Plate 2, Fig. 4, *x* and *y*) or may break right across apparently homogeneous tissue (Plate 2, Fig. 3). The difference between a deep-seated exogenous bud and an endogenous one is sometimes rather difficult to see (cf. Text Fig. 5A with Plate 2, Fig. 4) and cannot readily be seen in a single section. Serial sections, however, usually enable a distinction to be drawn between the closely adpressed outer walls of a deep depression and the newly separated cells of cracking tissue.

In 2-year old stems the orientation of the scars from the naked bud and the leaf petiole is slightly different from that of the younger nodes on the first year twigs.

Growth of the woody axis has forced them into a lateral position. Their relative positions as seen on surface view and in section are shown in Text Figure 6. Further growth of the stem alters their positions still more and eliminates the strands that served the deciduous axillary bud and the petiole. The third strand (*b* in Text Fig. 6), however, is persistent throughout the life of the tree. It is capped by an area of

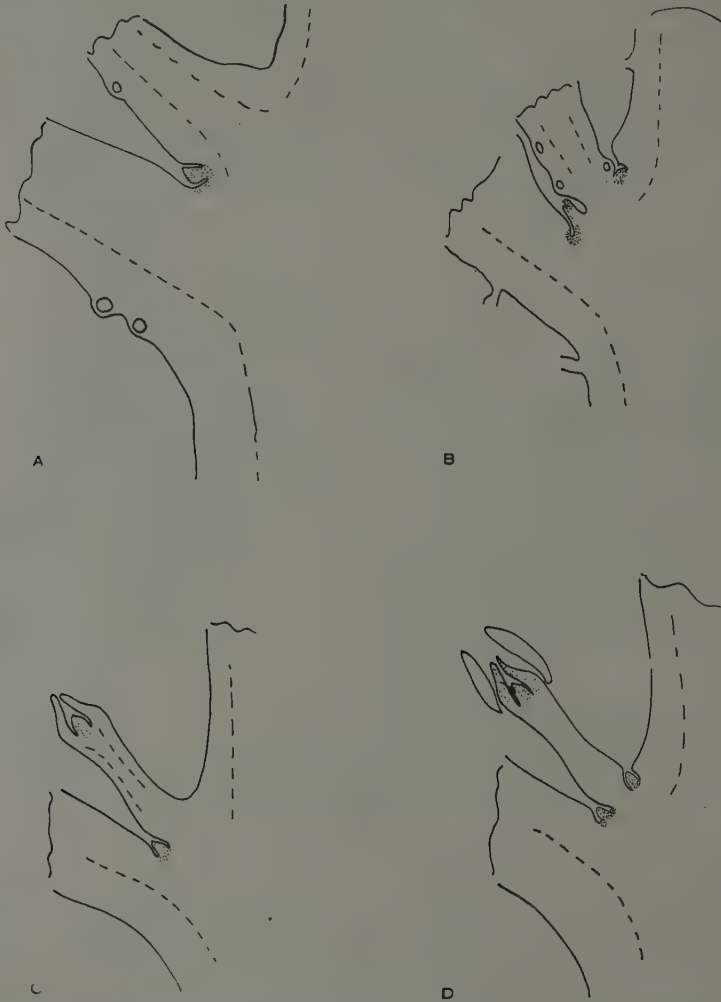


Fig. 7.—Nodes. *A* and *B*: *E. marginata*. *C* and *D*: *E. bicostata*. *A* and *C*: leaf nodes. *B* and *D*: cotyledonary nodes.

meristematic tissue and it grows at a pace that corresponds closely with that of the stem (Jacobs 1955). This strand, which soon pursues a direction almost at right angles to the stem axis, is the epicormic or dormant bud strand, and the meristematic area in the phloem immediately outside it is the site of any bud that is called into growth by defoliation of the tree by fire, injury, or insect attack to the crown. In older stems the leaf and shoot scars are eliminated by periderm formation, and the site of the

emergence of the epicormic strand is often hard to find, especially in the rough-barked species. On defoliation the factor inhibiting bud development is removed, and within a very short time a bud forms from the meristematic tissue capping the strand. The leaf veins soon develop within this bud and link it with the epicormic strand beneath it.

Although the most usual position for the concealed bud is between the naked bud scar and the petiole, exceptions to this can occasionally occur. Among the many nodes examined, three—one each of *E. eximia*, *E. maculata*, and *E. rudis*—showed a bud on the axial side of the naked bud scar as well as on the more usual side. Buds in similar positions on the lowest stem nodes, immediately above the cotyledons, also occur. They will be discussed in the following section. In material of *E. cladocalyx*, which had been repeatedly defoliated, the area from which proventitious buds arose became larger and, after many defoliations, buds were arising above as well as below and to the sides of the original leaf scar.

(d) Cotyledonary Nodes

The cotyledonary nodes of 47 species of *Eucalyptus* have been examined. Of these, 12 species are reported not to develop lignotubers.

It is at once apparent that there is a fundamental difference in anatomical structure between the cotyledonary nodes and the leaf node of the older stems. The cotyledonary nodes of 37 of the species examined consistently had concealed buds on both sides of the axillary shoot (Fig. 7), whereas the leaf nodes had concealed buds only below the axillary shoots, between them and the leaf petiole, except in the three species mentioned in the preceding section, and in the lowest nodes of the stem, immediately above the cotyledons. These buds are commonly exogenous, but are endogenous in some, though not all, of the species which have endogenous buds in the leaf nodes.

There is clearly a connexion between the capacity to produce lignotubers and this arrangement of the concealed buds, for the lignotubers, which in the first instance are merely swellings on the stem above the concealed buds, arise first and develop to a greater size on the upper side of the axillary shoot (Fig. 8), later spreading to the lower side and finally encircling the whole axillary shoot. This is of interest when it is considered in connexion with a statement of Jacobs (1955) that "if lignotubers are exposed by excavation, they tend to develop shoots from the lower part only. The lower part is really the younger, because the structures grow and bury themselves by enfolding down the root". This is indeed a continuance of the sequence of growth mentioned above, which started in the earliest stages of lignotuber formation from the concealed buds of the cotyledonary and lower stem nodes. Lignotubers do not arise, as the concealed buds do, from an area of meristematic tissue from which the leaves, stem, and ultimately the vascular tissue differentiate; they appear first as swellings of the stem above the axillary bud group (Figs. 8A–8F). Further growth comes from an extension of the cambium into this swelling. The meristems of the concealed buds are included in this swelling and further buds can develop around its periphery or deeper, within the cortical tissues that cover it. Other buds develop in the axils of the existing ones, thus giving rise to the clusters of buds which proliferate to form the

permanent bud tissue of the lignotubers. Growth of the connecting vascular strands keeps pace with the increasing girth of the lignotubers in the same way as the strands of the concealed buds on a stem keep pace with the increasing girth of the tree (Jacobs 1955). The buds on the lignotuber arise either exogenously at the base of the

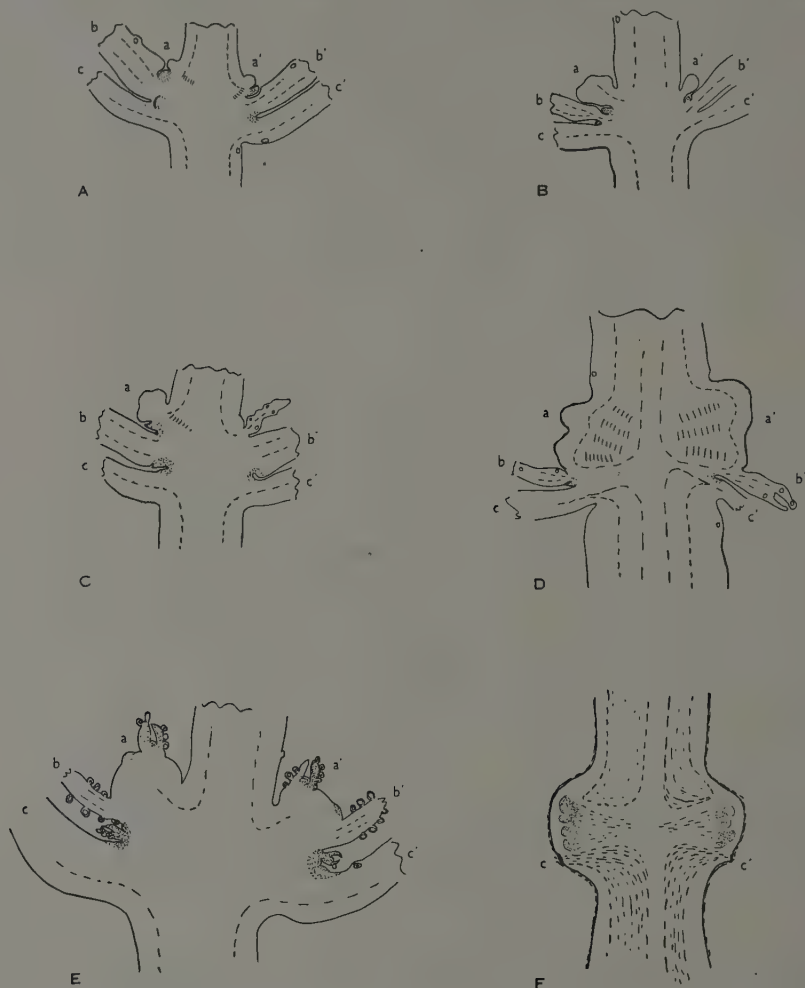


Fig. 8.—Young lignotubers. A and C: *E. leucoxyylon*. B and D: *E. viminialis*. E: *E. citriodora*. F: *E. polyanthemosa*. a, a', Young lignotubers; b, b', axillary shoot of cotyledonary node; c, c', cotyledons or cotyledonary abscission scar.

Further details are given in text.

existing buds (Fig. 8E) or endogenously from meristematic areas in the cortical or phloem tissue of the lignotubers (Fig. 8F).

The length of time that elapses before a lignotuber swelling appears on a seedling varies, not only from species to species, but also from season to season. The times given by Carter (1929) cannot be taken as invariable. Material grown for the present investigation in wintertime, even under glass, took several months to reach a stage

of development at which the concealed buds could be seen, while the same stage was reached by the same species in a few weeks in the summer. Lignotubers began to form correspondingly late in winter-grown material.

Lignotubers can form not only in the cotyledonary nodes but also in the first few nodes above the cotyledons. In many of the species examined nodes with buds on both sides of the axillaries were found among the lower stem nodes of small seedlings; they were not encountered in material from the upper parts of the stem of older seedlings. There is no doubt that had these seedlings grown to maturity lignotubers would have formed at the lower stem nodes as well as in the cotyledonary nodes.

This distinction between leaf and cotyledonary nodes, which is valid for the majority of *Eucalyptus* species examined, does not hold for 7 out of the 46 species. In *E. flocktoniae*, *E. fraxinoides*, *E. lehmanni*, *E. nitens*, *E. oreades*, and *E. regnans*, the cotyledonary nodes seldom differ from the leaf nodes. In 157 out of 172 cotyledonary and lower leaf nodes of these species there was only a single concealed bud, between the axillary bud and the cotyledonary or leaf petiole, instead of the double bud on both sides of the axillary shoot found in the other species examined.

One of the most peculiar species among those examined is *E. gigantea*, a non-tubering species. Not only are the upper concealed buds absent, but the lower ones, present in every node of every other species examined are also absent from the cotyledonary nodes. The first leaf node above the cotyledons has rudimentary concealed buds below the axillary buds. This species falls therefore into the category of species which lack the necessary mechanism for lignotuber formation and is additionally peculiar in having an apparently unique type of cotyledonary node.

V. DISCUSSION

As it has been established that growth above the axillary bud, involving the upper concealed bud, is necessary for the formation of lignotubers, it is not surprising to find that these swellings are absent from the seven species, named above, which lack the mechanisms necessary for their development. These species are, however, only a few of the eucalypt species from which lignotubers are known to be absent. Jacobs (1955) lists the following species: *E. astringens*, *E. camaldulensis*, *E. diversicolor*, *E. fastigata*, *E. gigantea*, *E. gomphocephala*, *E. grandis* (Hill) Maiden, *E. nitens*, *E. pilularis*, *E. regnans*, and *E. seeana* Maiden, and has recently (unpublished data) added *E. cloeziana* F. Muell. and *E. deglupta* Blume. From this, it would appear that, although lignotubers cannot be formed in the absence of the double set of concealed buds, the presence of this bud complex does not necessarily result in lignotuber formation. Among the species listed by Jacobs there are several in which the full bud complement is invariably present, but in which lignotubers never occur. The mallets of Western Australia are reported by Brockway and Hillis (1955) to occur both in tree and mallee forms, and these authors state that the occurrence of the various types is governed by site and, to some extent, by geographical conditions. It has not been possible to examine material of *E. falcata* Turcz or *E. spathulata* Hook., but material of *E. astringens* from other than the specialized sites mentioned by Brockway and Hillis, though possessing the double set of buds, has never been observed to make lignotubers. *E. gardneri*, on the other hand, is usually without the double set of buds

and would therefore be unlikely to make lignotubers, and only seed from the specially selected sites could prove whether these tuberizing mallee forms were anatomically similar to those commonly produced from commercially collected seed.

In some species which have been reported to be occasionally lignotuberous, notably *E. fastigata* and *E. camaldulensis* (Carter 1929), the provenance of the seed used for the investigation is obviously of great importance. Recent work by many different investigators has established the freely hybridizing qualities of many species of eucalypts and in all investigations in this genus seed of unknown origin can greatly affect the results. Material of *E. fastigata* from a commercial source, used in the present study, produced almost equal numbers of seedlings with the single and with the double bud complement and some seedlings with definite lignotubers. Yet, in sites where pure stands of *E. fastigata* are known to exist, hundreds of seedlings can be examined without any lignotubers being found. In adjacent areas, however, where hybridization with *E. robertsoni* Blakely is occurring, seedlings can be found which, while closely resembling the *E. fastigata* parent in most characters, are nevertheless lignotuberous and, to the expert eye, show other features which proclaim their hybrid origin.

It would appear that tuberous seedlings of *E. camaldulensis* are of similar doubtful parentage, for where pure stands can be examined the species is consistently non-tuberizing.

Kerr (1925) suggested that edaphic factors influenced tuber formation, but it would seem more probable that she was working with seed from localities where hybridization was possible. While site conditions cannot be entirely ruled out from a consideration of these non-tuberizing species, it seems far more likely that aberrations from the normal species behaviour are the result of working with seed of unknown origin. Jacobs (unpublished data) has found that maltreatment of the severest kind and growing in the most variable sites failed to produce tubers in pure strains of *E. camaldulensis*, nor does this species or *E. gomphocephala* produce tubers when it has been grown overseas in the Middle East or in Italy. Clearly some other factor than either the anatomical structure or environmental conditions must be looked for to explain the non-tuberizing of such species as *E. astringens*, *E. camaldulensis*, *E. pilularis*, and other species, in which the presence of the double set of buds in the cotyledonary and lower stem nodes provides the necessary anatomical mechanism for lignotuber formation.

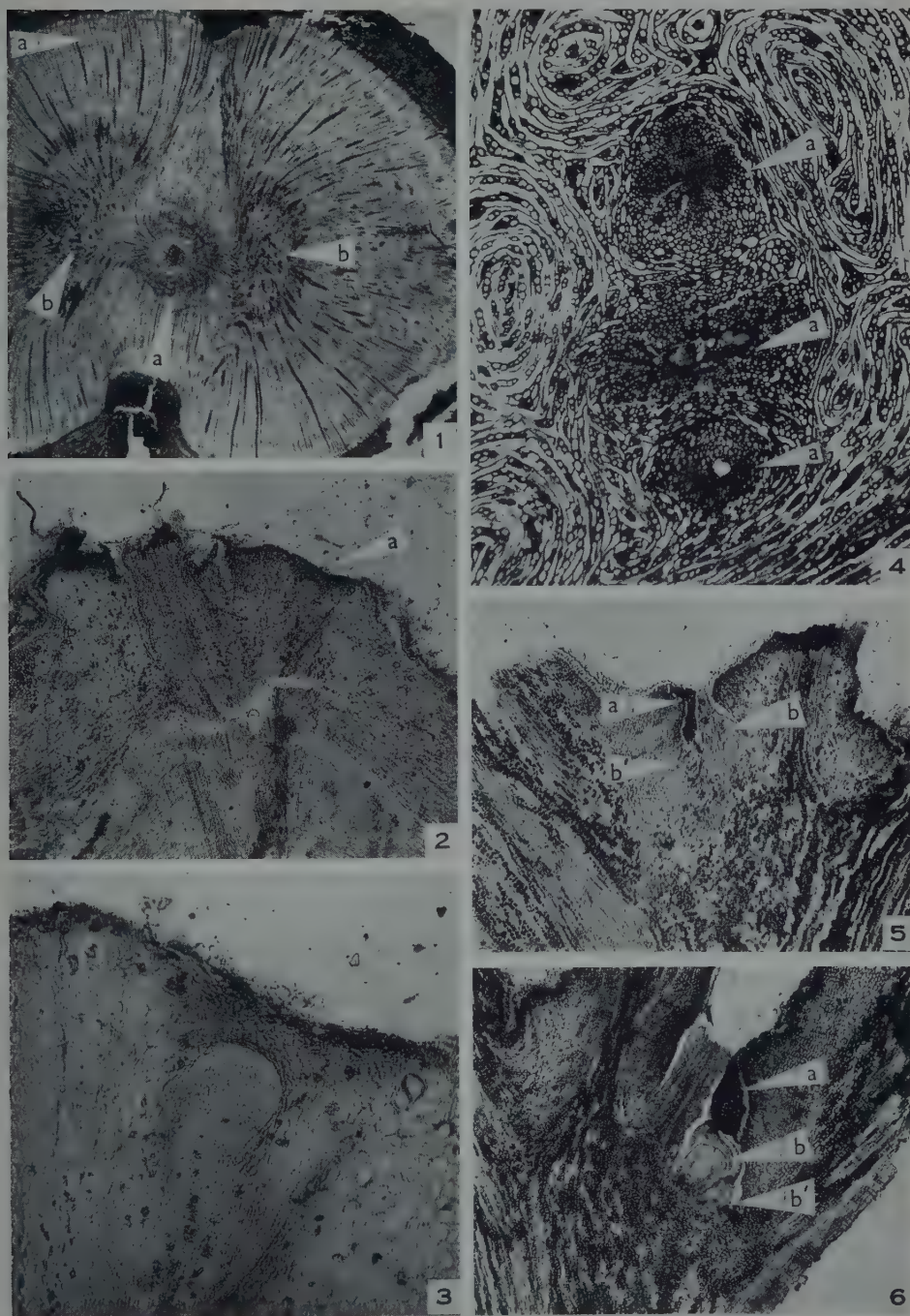
VI. ACKNOWLEDGMENTS

The author wishes to thank the Natural Resources Conservation League, Springvale South, Vic., for their kindness in providing a constant supply of seedlings for the investigation, and to Mr. L. D. Pryor for seeds and for encouragement and advice. She would also like to record the great patience of Miss M. Kemp and Miss H. Hewson, to whom fell the somewhat monotonous task of cutting the sections for the investigation.

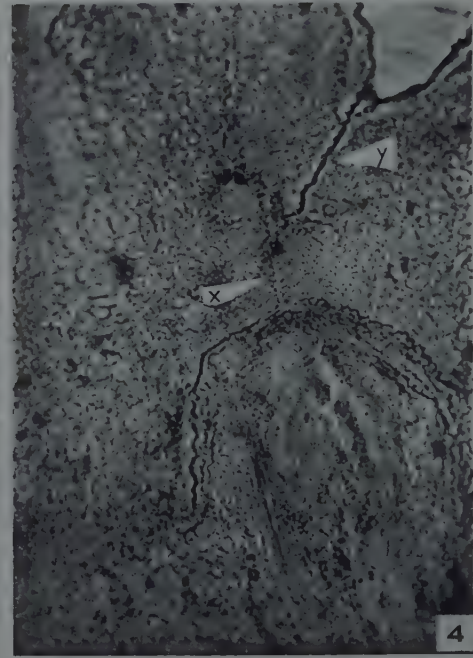
VII. REFERENCES

- BROCKWAY, G. E., and HILLIS, W. E. (1955).—*Emp. For. Rev.* **34** (1): 34–41.
CAREY, G. (1931).—*Proc. Linn. Soc. N.S.W.* **56**: 455–7.
CARTER, C. E. (1929).—*Aust. For. J.* **12**: 119–21.

BUD DEVELOPMENT AND LIGNOTUBER FORMATION IN EUCALYPTS



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- FOSTER, A. S. (1929).—*Amer. J. Bot.* **16**: 441-74; 475-501.
- JACOBS, M. R. (1936).—Commonwealth Forestry Bureau Bull. 18.
- JACOBS, M. R. (1955).—"Growth Habits of the Eucalypts." (Commonwealth of Australia, Forestry and Timber Bureau: Canberra.)
- KERR, L. R. (1925).—*Proc. Roy. Soc. Vict.* **37**: 79-97.
- WELCH, M. B. (1923).—*J. Roy. Soc. N.S.W.* **57**: 218-26.

EXPLANATION OF PLATES 1 AND 2

PLATE 1

- Fig. 1.—*E. viminalis*. Transverse section of part of young lignotuber, showing normal (*a*) and distorted (*b*) tissues merging into one another. $\times 18$.
- Fig. 2.—*E. melliodora*. Radial longitudinal section of portion of lignotuber. *a*, Site of endogenous bud. $\times 25$.
- Fig. 3.—*E. melliodora*. Portion of the above, at *a*, in greater detail. $\times 100$.
- Fig. 4.—*E. obliqua*. Radial longitudinal section of portion of lignotuber, showing tissue distortion and bud traces (*a*). $\times 45$.
- Fig. 5.—*E. maculata*. Longitudinal section through node, showing concealed buds (*b* and *b'*) with protective flap (*a*). $\times 20$.
- Fig. 6.—*E. maculata*. Longitudinal section through node, showing concealed buds (*b* and *b'*) with protective flap (*a*). $\times 20$.

PLATE 2

- Fig. 1.—*E. ficifolia*. Longitudinal section through node, showing concealed buds (*b*) with kino from protective flap (*a*) surrounding them. $\times 50$.
- Fig. 2.—*E. bicostata*. Longitudinal section through node, showing concealed bud (*b*), with protective flap on petiole (*a*), and base of axillary shoot (*c*). $\times 25$.
- Fig. 3.—*E. smithii*. Longitudinal section through endogenous bud, in advanced stage of growth, showing crack spreading through continuous line of darker tissue (*x*) to base of petiole and towards the axillary shoot. $\times 100$.
- Fig. 4.—*E. rudis*. Longitudinal section through endogenous bud, showing the beginning of a crack (*x*) which will connect with the base of the petiole and the axillary shoot and open as the bud expands. $\times 60$.

STUDIES ON THE ORIGIN, EVOLUTION, AND DISTRIBUTION OF THE GRAMINEAE

I. THE TRIBE ANDROPOGONEAE

By W. HARTLEY*

[*Manuscript received February 12, 1958*]

Summary

From a study of the grass flora of some 300 regions, a world distribution map of the grass tribe, Andropogoneae, has been prepared. This map shows that the Indo-Malaysian region is relatively rich in species of the tribe, with zones of high concentration in western India and in southern Indonesia. The relative species density falls off rapidly in passing from the tropical to the temperate zones, and is lower in the western than in the eastern hemisphere.

Climatic factors are of major importance in determining the distribution pattern. Winter temperatures are of special significance in temperate regions, while there is a striking relationship between high midsummer rainfall and relative abundance of species of Andropogoneae in the tropics and subtropics.

In general, the geographical survey supports conclusions drawn from taxonomic and cytological evidence regarding the origin and evolution of the tribe, but with greater emphasis on climate as a significant factor. The Andropogoneae have had a long evolutionary history in the eastern hemisphere, but have spread more recently to the western hemisphere, where they have not yet attained their full development.

I. INTRODUCTION

In previous publications (Hartley 1950, 1954) an index of relative specific differentiation was used as a basis for mapping the global distribution of the tribes of the Gramineae. This index is the number of species of grasses of a particular tribe in the flora of any region expressed as a percentage of the total number of grass species in that region. The distribution of tribes, mapped on this basis, showed relationship to climatic factors, and in particular to winter temperatures. This work has now been extended to include a more detailed study of the distribution of the larger grass tribes on a similar basis, beginning with the tribe Andropogoneae, which forms the subject of the present paper.

II. THE TRIBE ANDROPOGONEAE

With about 87 genera and 850 species (Pilger 1940, Potztl 1956) widely distributed, especially in the tropical and subtropical regions of both hemispheres, the Andropogoneae is one of the largest grass tribes. It is also taxonomically clearly delimited. While taxonomists differ in their views on generic limits, especially in the larger genera such as *Andropogon*, there is almost complete agreement on the limits of the tribe itself (Bews 1929; Avdulov 1931; Hubbard 1934; Keng 1939; Pilger 1940; Hitchcock 1951). The only exception to this unanimity concerns the desir-

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ability of including the Maydeae in the Andropogoneae. This is favoured by Celarier (1956) who considers the Maydeae to be a natural extension, through specialization, of the Andropogoneae. Because of the relatively few species of Maydeae, the incorporation of this tribe in the Andropogoneae would not substantially change the pattern of geographical distribution discussed in this paper.

During recent years several studies have added to our knowledge of the agronomy, morphology, and cytology of the Andropogoneae, including those by the authors listed above. Thus it is possible to compare the conclusions drawn from geographical studies with those derived from several other botanical disciplines. This fact, combined with the relative freedom from taxonomic complications, makes a study of the distribution of this tribe of special interest.

III. WORLD DISTRIBUTION OF THE ANDROPOGONEAE

The general distribution of the Andropogoneae throughout the world is shown in Figure 1.

In compiling this map, about 300 floras and floristic lists have been used, each of which is represented on the map by a point located at the approximate geographical centre of the region covered by the flora. The percentage of species of Andropogoneae in the total grass flora has been calculated for each of these regions, and lines have been drawn on the map to delimit those parts of the world with < 10, 10–20, 20–30, 30–40, and > 40 per cent. of species of Andropogoneae respectively.

All the readily available floras have been used in preparing the map, the only criteria for selection being:

- (a) the flora or list should have been published during the present century;
- (b) the list should be substantially complete for the grass flora of the region concerned, or, if the region is not well known floristically, should be free from bias towards particular tribes;
- (c) the regions covered by the floras and lists used should be sufficiently large to include at least 70 species of grasses, thus reducing minor statistical anomalies and giving an adequate representation of a range of ecological niches within the region concerned.

From examination of the map it will be evident that in most parts of the world the lists available have been adequate to permit a fairly clear definition of the frequency lines. The chief exceptions are in South America and in parts of southern and eastern Asia, where there is a lack of comprehensive modern floras. This is also true of many parts of Australia, but for this continent it has been possible to supplement the published information by utilizing unpublished lists of grasses from central and northern Australia and regional lists compiled from the grasses in the Queensland Herbarium.

The most conspicuous features of the general distribution map are (i) the concentration of the tribe in the tropical and subtropical parts of the world, (ii) the region of maximum abundance in Indo-Malaysia, and (iii) the relatively lower concentration in the western hemisphere than in the eastern hemisphere.

Each of these features is discussed in more detail below.

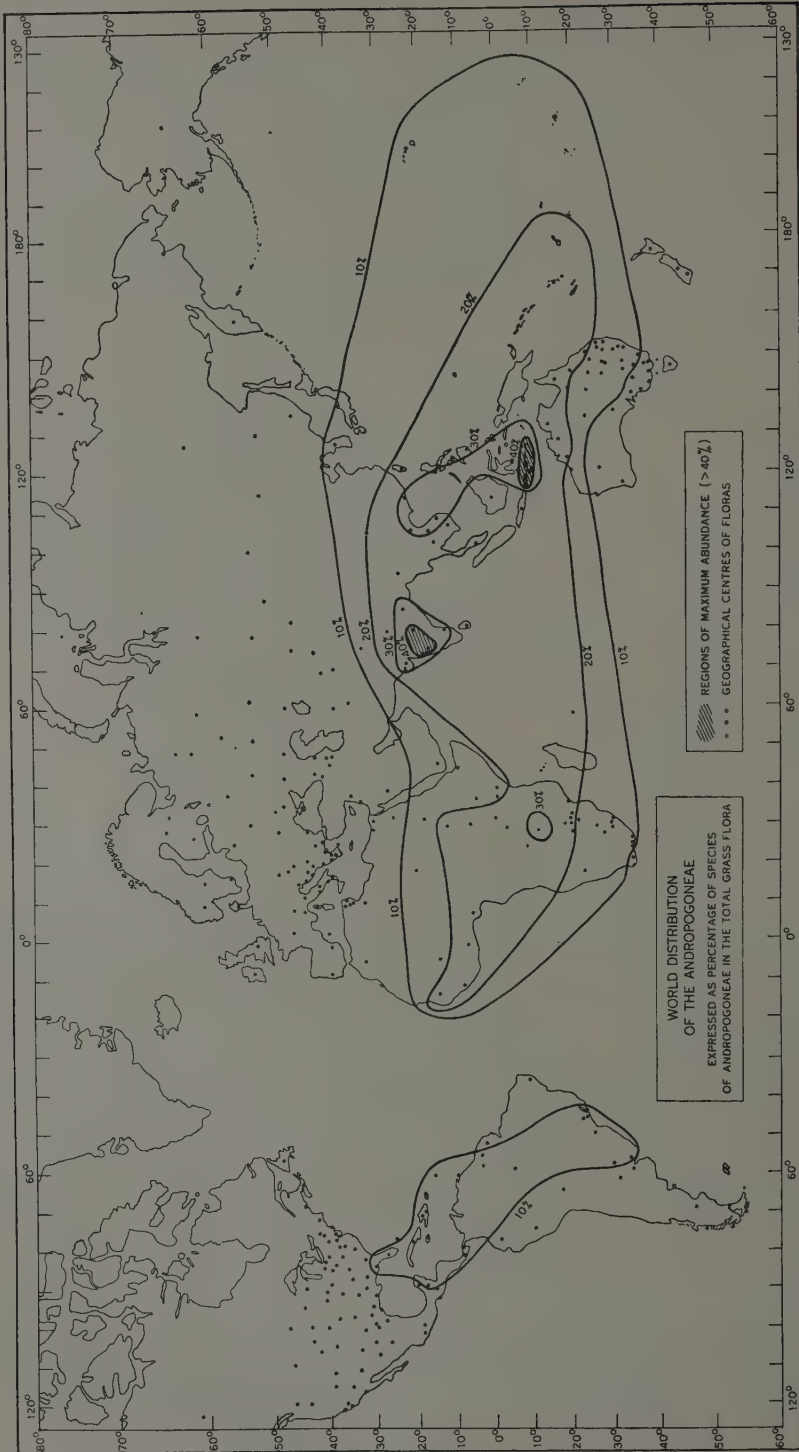


Fig. 1.—Map of world distribution of the Andropogoneae.

IV. REGIONS OF MAXIMUM CONCENTRATION

As shown by the map, most of the floras with a high percentage of species of *Andropogoneae* occur in southern Asia. With the single exception of the Katanga region of the Belgian Congo, all floras with more than 30 per cent. of *Andropogoneae* are in Indo-Malaysia, while five of these have more than 40 per cent. Details of the latter are given in Table 1, from which it will be noted that the highest concentrations are shown in two floras from the Western Ghats of India and one from the adjacent Bombay Deccan, with rather lower concentrations in the Lesser Sunda Islands and in Madhya Pradesh.

The eastern region of high concentration is not precisely delimited, because of the lack of adequate floristic information from some parts of Indonesia and especially from New Guinea. It is possible that the 30 per cent. zone should be extended eastwards to include the island of New Guinea. It is, however, clear that the regions

TABLE 1
REGIONS WITH HIGHEST PERCENTAGE OF SPECIES OF ANDROPOGONEAE

| Region | No. of Grass Spp. | | % of Androp. | Reference |
|---------------------------------|-------------------|---------|--------------|-------------------------|
| | Total | Androp. | | |
| Khandala, Western Ghats, India | 120 | 57 | 47.5 | Santapau 1953 |
| Western Ghats, India | 152 | 66 | 43.4 | Blatter and McCann 1935 |
| Bombay Deccan, India | 140 | 60 | 42.9 | Blatter and McCann 1935 |
| Lesser Sunda Islands, Indonesia | 143 | 61 | 42.7 | Kalkman 1955 |
| Madhya Pradesh, India | 207 | 88 | 42.5 | Tiwari 1954-55 |

of high concentration in India and Indonesia are separated by regions of lower concentration which include Assam, Siam, Cochin-China, the Malay Peninsula, Borneo, and Java. It is therefore of some interest to consider whether the two regions of high concentration are sufficiently distinctive floristically to indicate that they may be independent centres of distribution for the tribe.

Comparison of the genera and species of *Andropogoneae* which occur in Khandala, India (Santapau 1953) and in the Lesser Sunda Islands (Kalkman 1955) does not indicate that these regions are floristically independent. Each of the six subtribes of *Andropogoneae* recognized by Pilger (1940) is represented in both regions, with a strong predominance of the *Andropogoninae* (27 spp. in Khandala and 28 spp. in the Sunda Islands). Further, 14 genera are common to the two regions, a number which would be considerably increased by the adoption of a more conservative view of generic limits in the Indian flora.

The intermediate zone of lower concentration is probably adequately explained by climatic factors, which are discussed below. Also, since the concentration is expressed as the percentage of species of the tribe in the total grass flora, it is affected by the importance in this intermediate zone of another tribe, the *Bambuseae*. Each of the regions between Assam in the north and Java in the south has more than 10 per

cent. of bamboos in the total grass flora, with a maximum concentration in the Malay Peninsula (19.6 per cent.) and with lower percentages in the regions to the east and to

TABLE 2
DISTRIBUTION OF ANDROPOGONEAE ALONG LONGITUDE 30°E.

| Locality | Lat. | Long. | % of Androp. | Reference |
|---|--------|--------|--------------|---------------------------|
| Karelian Lapland, U.S.S.R. | 65°N. | 33°E. | 0.0 | Komarov 1934 |
| East Fennoscandia | 65°N. | 30°E. | 0.0 | Hylander 1955 |
| Finland | 63°N. | 26°E. | 0.0 | Hulten 1950 |
| Lagoda-Ilmen region, U.S.S.R. | 60°N. | 35°E. | 0.5 | Komarov 1934 |
| Baltic States | 56°N. | 25°E. | 0.0 | Hulten 1950 |
| Upper Dnieper region U.S.S.R. | 54°N. | 30°E. | 0.9 | Komarov 1934 |
| Middle Dnieper region, U.S.S.R. | 50°N. | 33°E. | 0.8 | Komarov 1934 |
| Northern Podolia, Ukraine | 49°N. | 28°E. | 1.1 | Motyka 1947 |
| Black Sea region, U.S.S.R. | 48°N. | 35°E. | 1.5 | Komarov 1934 |
| Dobruja region, Balkan Peninsula | 45°N. | 28½°E. | 4.6 | Hayek 1933 |
| Crimea, U.S.S.R. | 45°N. | 34°E. | 1.8 | Komarov 1934 |
| Bulgaria | 43°N. | 25°E. | 3.1 | Hayek 1933 |
| E. Macedonia and W. Thrace | 41°N. | 25°E. | 3.1 | Rechinger 1939 |
| Thrace | 41°N. | 26½°E. | 3.9 | Hayek 1933 |
| Cyclades Is. | 37°N. | 25°E. | 5.6 | Hayek 1933 |
| Crete | 35°N. | 25°E. | 5.0 | Hayek 1933 |
| Mediterranean Egypt | 31°N. | 29°E. | 4.5 | Muschler 1912 |
| Nile Delta region, Egypt | 31°N. | 31°E. | 9.5 | Muschler 1912 |
| Desert region, Egypt | 26°N. | 32°E. | 10.0 | Muschler 1912 |
| Northern region, Sudan | 19°N. | 31°E. | 18.2 | Andrews 1956 |
| Central region, Sudan | 13°N. | 29°E. | 21.0 | Andrews 1956 |
| Southern region, Sudan | 7°N. | 30°E. | 22.5 | Andrews 1956 |
| Uganda | 1°N. | 32°E. | 21.6 | Maitland and Hubbard 1927 |
| Uganda | 1°N. | 32°E. | 20.5 | Eggeling 1947 |
| Ruzizi Plain, Belgian Congo | 3°S. | 29°E. | 23.3 | Germain 1952 |
| Katanga, Belgian Congo | 11°S. | 28°E. | 30.3 | de Wildeman 1921 |
| Northern Division, S. Rhodesia | 17°S. | 30°E. | 24.1 | Sturgeon 1953-56 |
| Southern Rhodesia | 19°S. | 30°E. | 22.6 | Stent and Rattray 1933 |
| Central Division, S. Rhodesia | 19°S. | 31°E. | 21.9 | Sturgeon 1953-56 |
| Eastern Division, S. Rhodesia | 19°S. | 32½°E. | 23.3 | Sturgeon 1953-56 |
| Mozambique | 19°S. | 35°E. | 27.9 | José Gomes Pedro 1954 |
| Western Division, S. Rhodesia | 19½°S. | 28°E. | 21.4 | Sturgeon 1953-56 |
| Southern Division, S. Rhodesia | 21°S. | 31°E. | 19.3 | Sturgeon 1953-56 |
| Transvaal, S. Africa | 25°S. | 29°E. | 19.6 | Stent 1924 |
| Potchefstroom region, Transvaal | 27°S. | 27°E. | 16.8 | Louw 1951 |
| Leribe Plateau, Basutoland | 29°S. | 29°E. | 17.0 | Phillips 1917 |
| Natal and Zululand | 29°S. | 31°E. | 17.4 | Bews 1921 |
| Weenen County, Natal | 29°S. | 30°E. | 18.4 | West 1951 |
| Uitenhage and Port Elizabeth, S. Africa | 34°S. | 25°E. | 11.9 | Schonland 1919 |

the west. As the zone of high concentration of Bambuseae coincides precisely with the intermediate zone of lower concentration of Andropogoneae, the two are evidently interrelated.

In general, Indo-Malaysia can be regarded as a region of high relative concentration of Andropogoneae, within which are local variations determined by special factors.

V. CLIMATIC FACTORS IN RELATION TO DISTRIBUTION*

As indicated previously (Hartley 1950), there is evidence that climatic factors, including winter temperature and possibly rainfall, are of importance in relation to the distribution of the Andropogoneae. This is supported by cursory examination of Figure 1. The effect of temperature is reflected in the predominantly tropical and subtropical distribution of the tribe, while its abundance in different parts of the tropical zone appears to be influenced by rainfall.

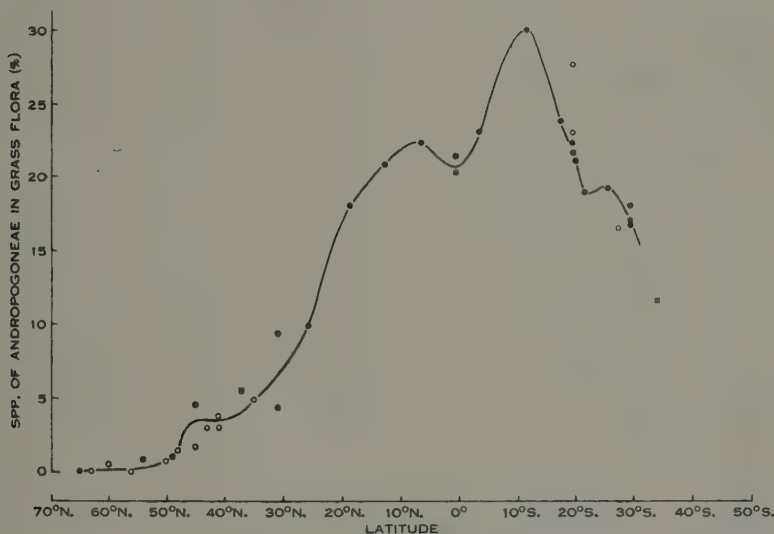


Fig. 2.—Percentage of species of Andropogoneae in grass flora along longitude 30°E.
●, Region with geographical centre between 28°E. and 32°E. ○, Region with geographical centre between 25°E. and 35°E. but not between 28°E. and 32°E.

In order to investigate this relationship more fully, a study has been made of the frequency of the tribe within a longitudinal transect bounded by meridians 25°E. and 35°E. This transect is particularly suitable for the purpose as it covers a wide latitudinal range of land areas, from Lapland in the north to South Africa in the south, and sufficient floristic lists are available to permit adequate definition of the distribution pattern.

In Table 2 the regions for which floristic data are available are listed in order from north to south, with the approximate geographical centre and the percentage

*In addition to other references mentioned in the text the following sources have been used for climatic data: Central Geophysical Observatory (1926); Ministry of Public Works, Egypt (1938); Nuttonson (1947, 1950); Tothill (1952); Union of South Africa (1954); and East African Meteorological Department (undated); together with unpublished data supplied by the Directors of the Meteorological Services of Southern Rhodesia and of the Belgian Congo, and by the Department of Agronomy, University of Ankara, Turkey.

of species of *Andropogoneae* in the grass flora of each region. It will be noted that some of the regions overlap, while Uganda is covered by two floras, prepared by different authorities at different times.

TABLE 3
TEMPERATURE AND RAINFALL ALONG LONGITUDE 30°E.

| Lat. | Midwinter Temp.* (°F) | Midsummer Rainfall† (in.) | Lat. | Midwinter Temp. (°F) | Midsummer Rainfall (in.) |
|-------|-----------------------------|---------------------------------|-------|----------------------------|--------------------------------|
| 69°N. | 18 | 2.1 | 17°N. | n.d.‡ | n.d. |
| 67°N. | 13 | 2.5 | 15°N. | n.d. | n.d. |
| 65°N. | 11 | 2.9 | 13°N. | 70 | 4.5 |
| 63°N. | 14 | 2.6 | 11°N. | n.d. | 6.3 |
| 61°N. | 18 | 2.7 | 9°N. | 80 | 6.7 |
| 59°N. | 18 | n.d. | 7°N. | 80 | 5.9 |
| 57°N. | 18 | n.d. | 5°N. | 81 | 6.3 |
| 55°N. | 18 | n.d. | 3°N. | 73 | 6.3 |
| 53°N. | 19 | n.d. | 1°N. | 72 | 3.6 |
| 51°N. | 21 | 3.2 | 1°S. | 65 | 3.3 |
| 49°N. | 21 | 2.8 | 3°S. | 67 | 4.8 |
| 47°N. | 25 | 1.8 | 5°S. | 71 | 5.1 |
| 45°N. | n.d. | n.d. | 7°S. | 68 | 6.3 |
| 43°N. | n.d. | n.d. | 9°S. | 63 | 8.9 |
| 41°N. | 41 | 2.4 | 11°S. | 62 | 10.9 |
| 39°N. | 33 | 0.8 | 13°S. | 61 | 11.0 |
| 37°N. | 43 | 0.2 | 15°S. | 63 | 8.5 |
| 35°N. | n.d. | n.d. | 17°S. | 58 | 8.4 |
| 33°N. | n.d. | n.d. | 19°S. | 57 | 7.2 |
| 31°N. | 54½ | 0.0 | 21°S. | 58 | 5.5 |
| 29°N. | 53 | 0.0 | 23°S. | 57 | 7.4 |
| 27°N. | 52 | 0.0 | 25°S. | 53 | 5.6 |
| 25°N. | 56 | 0.0 | 27°S. | 49 | 5.2 |
| 23°N. | n.d. | n.d. | 29°S. | 54 | 5.3 |
| 21°N. | 61 | 0.0 | 31°S. | 55 | 4.5 |
| 19°N. | 69 | 0.3 | | | |

* "Midwinter temperature" is mean January temperature for northern latitudes and mean July temperature for southern latitudes.

† "Midsummer rainfall" is mean July rainfall for northern latitudes and mean January rainfall for southern latitudes.

‡ n.d. indicates no data.

From inspection of the table, it is evident that there is a relationship between the frequency of species of the tribe and latitude. This is more clearly apparent from Figure 2 in which the data have been plotted and a curve fitted to them. In this figure the floras with a geographical centre lying between longitudes 28°E. and 32°E. are indicated in solid black, while those lying between 25°E. and 35°E. but not within the narrower longitudinal zone are shown by open circles. It will be noted that, in general, the curve is well defined by the available reference points, especially if special attention is given to those floras which most closely approach the 30°E. meridian.

The only points which deviate from the curve are those for the Mediterranean and Nile Delta regions of Egypt, shown at latitude 31°N. , and one for Mozambique, at 19°S. The Egyptian data are affected by the great importance of irrigation along the Nile valley, which necessarily affects the grass flora, while Mozambique lies wholly to the east of meridian 30°E. and is predominantly a lowland area with higher temperatures than places at corresponding latitudes along the 30°E. meridian.

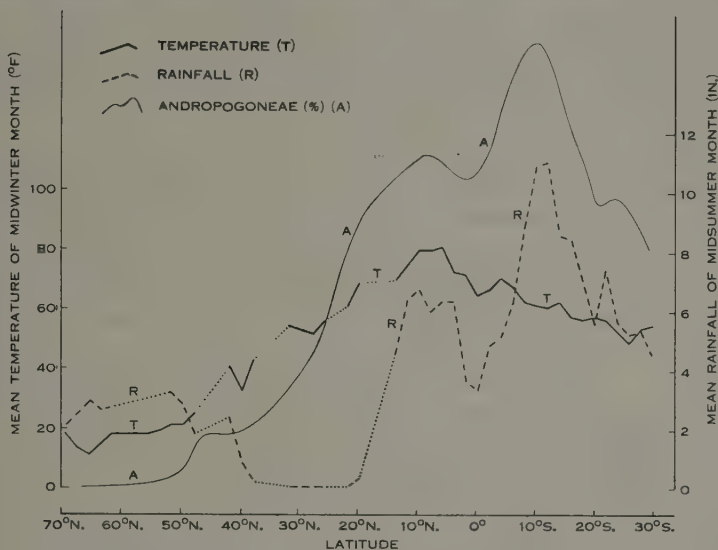


Fig. 3.—Comparison of distribution of *Andropogoneae* with midwinter temperature and midsummer rainfall along longitude 30°E.

The curve shows the following conspicuous features:

- (1) a progressive increase in the percentage of species of *Andropogoneae* in moving southwards from high northern latitudes to about 8°N. ;
- (2) a fall in percentage from 8°N. to around the equator;
- (3) a rise from the equator to a major peak around 11°S. ;
- (4) a fall in proceeding southwards from 11°S. , with a probable minor upturn between 21°S. and 24°S. ;
- (5) consistently higher values for the percentage of *Andropogoneae* south of the equator in comparison with corresponding northern latitudes.

Data for mean midwinter (January or July) temperatures and mean midsummer rainfall for the same longitudinal transect are shown in Table 3. In compiling this table the temperature or rainfall given for any latitude x° is the mean of the values for all available recording stations bounded by latitudes $x^{\circ}-1^{\circ}$ and $x^{\circ}+1^{\circ}$, and by longitudes 28°E. and 32°E. The values are thus reasonably representative of areas corresponding to the floristic lists. For some latitudes no data were available, usually because the particular latitudinal intersection corresponds to sea rather than to land, or because it is in a desert region without recording stations.

The data are shown graphed in Figure 3, with the curve of grass distribution from Figure 2 inserted for comparison.

If comparison is made between the graph for mean temperature of the mid-winter month (T) and that for percentage of species of *Andropogoneae* (A), similarities of trend are apparent in the northern hemisphere. With midwinter temperatures below 20°F species of *Andropogoneae* are absent or form a negligible proportion of the grass flora. The percentage increases with rising midwinter temperatures south of latitude 50°N., reaching a minor peak at the zone of highest temperature between 5°N. and 10°N. However, there is no corresponding agreement between the two curves south of the equator. The progressive fall in the temperature curve contrasts sharply with the rise in the grass curve to its peak around 11°S., and it is evident that some factor other than winter temperature is of importance.

There are strong indications that summer moisture is important in relation to grass distribution south of the equator, and, indeed, throughout the tropical and subtropical zone. Comparison of curve A with that of mean rainfall for the mid-summer month (R) shows striking similarities of trend for all latitudes south of 20°N. The conspicuous features of the grass frequency curve, listed as (2) to (4) above, are all equally evident in the graph of summer rainfall, including even the upturn south of 21°S. Also, except immediately around the equator, the summer rainfalls are higher in southern than in corresponding northern latitudes, again paralleling the situation shown by the grass frequency curve. It is only in regions of low winter temperature that summer rainfall has no apparent significance.

It would be unwise to overstress these comparisons, for the areas from which floristic and climatic data have been obtained do not exactly correspond, and it is evident that the rainfall of the midsummer month is a very inadequate indication of the moisture available for plant growth during the hottest period of the year. Nevertheless, it is of interest to note that the conclusions drawn from this survey of the distribution of the *Andropogoneae* within a narrow longitudinal transect are supported by data from other parts of the tropical zone of the eastern hemisphere. Thus those regions which show an apparently anomalous low percentage of species of *Andropogoneae* are characterized either by high altitude and consequential low winter temperatures (e.g. the Kenya highlands) or by low summer rainfall (e.g. western British Somaliland). Conversely, it is noteworthy that the Khandala region of India, which has the highest recorded percentage of species of the tribe (Table 1), has an extreme monsoonal climate with several rainless months but with a midsummer (July) rainfall of no less than 85 in. (Santapau 1953).

A similar relationship between the distribution of *Andropogoneae* and climatic factors is apparent in the western hemisphere, as judged by a comparison of the distribution map (Fig. 1) with the small-scale temperature and rainfall maps given in the Oxford Advanced Atlas. Again, the regions of low frequency within the tropical zone are either those of high altitude (e.g. Bolivia) or of relatively low mid-summer rainfall (e.g. Pernambuco, Brazil). However, the tribe is less abundant in the grass flora throughout the western hemisphere than would be expected if climate were the sole important factor. As previously indicated (Hartley 1950), and more fully discussed below, there is evidence that the *Andropogoneae* have not yet reached their full potential development in the western hemisphere.

VI. GEOGRAPHICAL DISTRIBUTION AND EVOLUTIONARY HISTORY

The pattern of geographical distribution of the tribe Andropogoneae described in previous sections of this paper may be considered in the light of evidence about its evolution derived from cytological and other studies.

It has been noted that the main centre of specific differentiation is in southern Asia. This region is also rich in taxa which have been regarded, both by taxonomists and cytologists, as being the most primitive members of the tribe. These include the subtribe Saccharinae and especially the genus *Miscanthus*, which not only has a generalized type of inflorescence from which the more specialized forms within the tribe may have been derived, but which also shows relationships to other tribes of grasses (Avdulov 1931; Keng 1939; Celarier 1956). The Saccharinae are also typically tall, hygrophilous grasses, which are considered to be ecologically primitive (Bews 1929). It has been shown in the present paper that the general adaptation to hot, moist conditions is maintained throughout the tribe.

However, the strong evidence that comparative specific differentiation in the tribe is closely associated with certain climatic factors suggests that care must be exercised in drawing conclusions about centres of origin and evolution from data of this kind. While the percentage of species of Andropogoneae in the flora of Africa does not anywhere approach the high levels reached in some parts of southern Asia, this may well be due to the absence in Africa of comparable levels of summer rainfall and is not necessarily to be accepted as evidence that the tribe has spread into Africa from a centre of origin in Asia. In fact, the evidence suggests that such a spread, if it occurred at all, took place during the very early history of the tribe. The presence in tropical Africa of the genus *Miscanthidium*, closely related to, or perhaps congeneric with *Miscanthus*, indicates that the primitive forms of the tribe reached Africa at a very early stage, if they did not, indeed, originate there. It is probable that somewhat parallel evolution and spread occurred in Africa and in Asia, with the tribe throughout retaining its tendency to develop maximum differentiation in regions of high winter temperature and abundant summer moisture. Although there is some evidence, as noted by Keng (1939), that "ecologically the more advanced genera are more adapted to drier and cooler conditions", their development has not proceeded to such a stage as to blur the general picture of maximum differentiation under hot, moist conditions. In fact, the present distribution of the Andropogoneae in the eastern hemisphere, on the basis used in this paper, can be fully explained in terms of present climatic factors.

The situation is different if the distribution of the Andropogoneae in the western hemisphere is compared with that in the eastern hemisphere. In the American continent, species of the tribe are throughout less numerous in comparison with other grasses than would be expected from climatic considerations alone. This suggests strongly that the tribe has not reached its full potential development in this region and that it has spread to America from a centre or centres of origin in the Old World. This conclusion is reinforced by taxonomic evidence. Not only are the more primitive taxa of the tribe absent from the American continent, but it is noteworthy that only one of the 87 genera (*Agenium*) is recorded as endemic there. The subtribe Dimeriinae and the groups Eulaliinae and Vossiinae are not represented in America

(Pilger 1940). Inclusion of the tribe Maydeae in the Andropogoneae would give a greater relative representation in the western hemisphere, including two or three additional endemic genera. It would not, however, affect the general evidence that the Andropogoneae have reached the American continent at a late stage of evolutionary development.

In general, therefore, the geographical studies reported here support taxonomic and cytological conclusions about the origin and evolution of the tribe Andropogoneae, while emphasizing the importance of climate as a major factor in the present distribution pattern. They also support the conclusions reached in a previous study (Hartley 1950), but show that there is a close relationship between high relative specific differentiation of the tribe in the tropical regions and high midsummer rainfall.

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VIII. REFERENCES

- ANDREWS, F. W. (1956).—"The Flowering Plants of the Anglo-Egyptian Sudan." Vol. 3, p. 579. (T. Buncle & Co. Ltd.: Arbroath.)
- AVDULOV, N. P. (1931).—Karyo-systematische Untersuchung der Familie Gramineen. *Bull. Appl. Bot. Pl. Breed. Suppl.* **43**: 428.
- BEWS, J. W. (1921).—"Flora of Natal and Zululand." p. 248. (City Printing Works: Pietermaritzburg.)
- BEWS, J. W. (1929).—"The World's Grasses." p. 408. (Longmans, Green & Co.: London.)
- BLATTER, E., and McCANN, C. (1935).—"The Bombay Grasses." p. 324. (Imperial Council of Agricultural Research: Delhi.)
- CELARIER, R. P. (1956).—Cytotaxonomy of the Andropogoneae I. Subtribes Dimeriinae and Saccharinae. *Cytologia* **21**: 272-91.
- CENTRAL GEOPHYSICAL OBSERVATORY (1926).—"Climate of the Union of Soviet Socialist Republics." (In Russian.) p. 128. (Leningrad.)
- EAST AFRICAN METEOROLOGICAL DEPARTMENT (undated).—Collected climatological statistics for East African stations. p. 44. (Nairobi; mimeographed.)
- EGGELING, W. J. (1947).—"An Annotated List of the Grasses of the Uganda Protectorate." p. 54. (Government Printer: Entebbe.)
- GERMAIN, R. (1952).—Les associations végétales de la Plaine de la Ruzizi (Congo Belge) en relation avec le milieu. Publ. Inst. Nat. Agron. Congo. Belge, Ser. Sci. No. 52, p. 321.
- HARTLEY, W. (1950).—The global distribution of tribes of the Gramineae in relation to historical and environmental factors. *Aust. J. Agric. Res.* **1**: 355-73.

- HARTLEY, W. (1954).—The agrostological index: a phytogeographical approach to the problems of pasture plant introduction. *Aust. J. Bot.* **2**: 1–21.
- HAYEK, A. (1933).—"Prodromus Florae Peninsulae Balcanicae. 3. Band. Monocotyledoneae." *Repert. Nov. Spec. Regn. Veg. Beihefte, Band XXX*, **3**: 1–472.
- HITCHCOCK, A. S. (1951).—"Manual of the Grasses of the United States." (Revised by A. Chase.) p. 1051. (United States Government Printing Office: Washington.)
- HUBBARD, C. E. (1934).—Gramineae. In "The Families of Flowering Plants. II. Monocotyledons." By J. Hutchinson. p. 243. (Macmillan & Co. Ltd.: London.)
- HULTEN, E. (1950).—"Atlas of the Distribution of Vascular Plants in N.W. Europe." p. 512. (Generalstabens Litografiska Anstalts Förlag: Stockholm.)
- HYLANDER, N. (1955).—"List of the Plants of N.W. Europe. I. Vascular Plants." p. 175. (CWK Gleerups Förlag: Lund.)
- JOSÉ GOMES PEDRO (1954).—Contribuições para a inventario florístico de Moçambique. *Bol. Soc. Estud. Moçambique* **24**: 1–87.
- KALKMAN, C. (1955).—A plant-geographical analysis of the Lesser Sunda Islands. *Acta Bot. Neerl.* **4**: 200–25.
- KENG, Y. L. (1939).—The gross morphology of Andropogoneae. *Sinensia* **10**: 274–343.
- KOMAROV, V. L. (1934).—"Flora Unionis Rerumpublicarum Sovieticarum Socialisticarum." (Acad. Sci. U.R.S.S.: Moscow.)
- LOUW, W. J. (1951).—An ecological account of the vegetation of the Potchefstroom area. *Mem. Bot. Surv. S. Afr. No. 24*, p. 105.
- MAITLAND, T. D., and HUBBARD, C. E. (1927).—Uganda grasses. *Kew Bull.* **1927**: 272–305.
- MINISTRY OF PUBLIC WORKS, EGYPT (1938).—"Climatological Normals for Egypt and the Sudan, Cyprus and Palestine." p. 148. (Cairo.)
- MOTYKA, J. (1947).—La distribution et l'écologie des plantes vasculaires sur la limite septentrionale de la Podolie occidentale. *Ann. Univ. M. Curie-Sklodowska, Sect. C, Suppl. III*, p. 400.
- MUSCHLER, R. (1912).—"A Manual Flora of Egypt." p. 1312 (R. Friedlander und Sohn: Berlin.)
- NUTTONSON, M. Y. (1947).—Ecological crop geography of the Ukraine. *Amer. Inst. Crop Ecol., Intern. Agro-climatic Series No. 1*.
- NUTTONSON, M. Y. (1950).—Ecological crop geography of Finland. *Amer. Inst. Crop. Ecol., Intern. Agro-climatic Series No. 10*.
- PHILLIPS, E. P. (1917).—Contribution to the flora of the Leribe Plateau and environs (Basutoland). *Ann. S. Afr. Mus.* **16**: 1–379.
- PILGER, R. (1940).—Gramineae III (Unterfam. Panicoideae). In "Die Natürlichen Pflanzenfamilien." By A. Engler and K. Prantl. Vol. 14e, p. 208. (Wilhelm Engelmann: Leipzig.)
- POTZTAL, E. (1956).—Nachtrag zu Gramineae III. In "Die Natürlichen Pflanzenfamilien." By A. Engler and K. Prantl. Vol. 14d, p. 225. (Duncker und Humblot: Berlin.)
- RECHINGER, K. H. (1939).—Zur Flora von Ostmazedonien und Westthrazien. *Bot. Jb.* **69**: 419–552.
- SANTAPAU, H. (1953).—Flora of Khandala on the Western Ghats of India. *Rec. Bot. Surv. India* **16** (1): 1–396.
- SCHONLAND, S. (1919).—The phanerogamic flora of Uitenhage and Port Elizabeth. *Mem. Bot. Surv. S. Afr. No. 1*, p. 118.
- STENT, S. M. (1924).—Grasses of the Transvaal as represented in the National Herbarium. *Bothalia* **1**: 222–303.
- STENT, S. M., and RATTRAY, J. M. (1933).—The grasses of Southern Rhodesia. *Proc. Rhod. Sci. Ass.* **32**: 1–64.
- STURGEON, K. E. (1953–56).—A revised list of the grasses of Southern Rhodesia. *Rhod. Agric. J.* **50**: 278–91, 420–39, 497–514; **51**: 12–27, 131–45, 212–26, 293–312, 379–93, 495–508; **52**: 39–63; **53**: 110–41.

- TIWARI, S. D. N. (1954-55).—The grasses of Madhya Pradesh. *Indian For.* **80**: 601-11, 681-9; **81**: 107-15, 191-200.
- TOTHILL, J. D. (1952).—“Agriculture in the Sudan.” 2nd impress. p. 974. (Oxford Univ. Press.)
- UNION OF SOUTH AFRICA (1954).—Official Yearbook No. 27 for 1952/53.
- WEST, O. (1951).—The vegetation of Weenen County, Natal. *Mem. Bot. Surv. S. Afr.* No. 23, p. 183.
- DE WILDEMAN, E. (1921).—“Contribution à l'Étude de la Flore du Katanga.” p. 264. (Brussels.)

SOME LABORATORY GERMINATION RESPONSES OF THE SEEDS OF
RIVER RED GUM, *EUCALYPTUS CAMALDULENSIS* DEHN. SYN.
EUCALYPTUS ROSTRATA SCHLECHT.

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Summary

A method for sampling from bulked eucalypt seed for germination tests, and a special technique for testing germination of these seeds in Petri dishes are described.

The seeds of *Eucalyptus camaldulensis* Dehn. will germinate both at constant and at alternating temperatures. They germinate best at a constant temperature of about 95°F.

The seeds require light for satisfactory germination. The light requirements of all seed-lots tested were satisfied by 8 hours of light daily at a constant temperature of 95°F. Light requirements are least at temperatures close to 95°F. There is an interaction between light and temperature and the requirement for light is reduced as the temperature is increased. Stratification also reduces the requirement for light in germination.

Thirty-one different lots of seeds have been studied. It has been shown that no relationship exists between environmental conditions of the locality of collection and optimum conditions for germination.

I. INTRODUCTION

Seed-testing to obtain information on purity, viability, and optimum conditions for germination is standard practice in agriculture and forestry. However, very little work of this kind has been done with species of the genus *Eucalyptus* and the only data available on the typical germination responses of *Eucalyptus* seeds under different conditions of constant and alternating temperatures and of light and darkness appear to be those of Clifford (1953).

It has been found necessary to devise special techniques to study the reactions of *Eucalyptus* seeds. These special techniques were required because the number of seeds in a given sample is always much smaller than the number of sterile ovules or "chaff".

Preliminary trials indicated that seeds of some species of *Eucalyptus* germinate well at constant temperature but only within the limits of a narrow temperature range. This phenomenon, coupled with the wide climatic tolerance of certain species, suggested a possible relationship between the environmental conditions of the locality of collection and optimum conditions for germination. However, the scope of the preliminary trials was not wide enough to establish that the responses of seeds of a given species did or did not vary whether collected from trees in different localities or even from adjacent trees. It was therefore necessary to examine the responses of different collections of seeds of a single species to discover whether they displayed any

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lack of uniformity, before it was possible to embark on an investigation of the environmental or other causes of any non-uniform behaviour.

In the course of the investigation to be described below the basic requirements for germination of a single species of *Eucalyptus* have been examined and the germination responses of seed-lots collected from different parent trees of this species have been compared. *E. camaldulensis* Dehn. was chosen because of its extremely wide distribution. Six lots of seed were collected at intervals along the Murray River between Mildura and Tallangatta, an air-line distance of approximately 300 miles. In order to extend the range of localities seed was also collected from four non-riverine areas (Fig 1.). Germination responses of seeds collected from trees a few miles apart were also tested as well as those from individual trees growing close together, and from the north, south, east, and west sides of a single tree.



Fig. 1.—Map of Victoria showing localities from which *E. camaldulensis* seeds were collected.

II. MATERIALS AND METHODS

(a) Capsule Collection, Seed Extraction and Cleaning

Capsules were picked from felled or standing trees, and the seeds were extracted from the capsules by spreading them on canvas or trays to facilitate rapid drying. The seeds were then readily extracted by vigorously shaking the opened capsules. A graded series of sieves was used to remove impurities such as grit, twigs, leaves, etc. The samples referred to as seed consisted of fertile seeds and "chaff".

(b) Provenance of Seed-lots

Thirty-one seed-lots were used in the investigation. These came from riverine, non-riverine, and savannah woodland type stands (Table 1). Lots 1–6 from along the

Murray River constituted the first riverine series. Lots 10–13 from savannah woodland type near Skipton were from trees approximately 3 miles apart in a line along the Ballarat–Hamilton highway. Lots 14–16 and 17–19 were collected from two groups each of three trees approximately 25 miles apart in the Mildura district, while lots 20–23 and 24–27 were collected from two groups each of four trees, 12 miles apart in the Barmah district. Lots 28–31 were collected from the north, south, east, and west sides of one tree.

TABLE 1
COLLECTION DATA FOR THE SEED-LOTS USED IN THE STUDY

| Lot No. | Locality | Date of Collection | Forest Type | Number of Trees Sampled per Seed-lot |
|---------|-------------|--------------------|-------------------|---|
| 1 | Swan Hill | Nov. 1952 | Riverine | Several |
| 2 | Gunbower | Aug. 1953 | Riverine | Several |
| 3 | Barmah | July 1953 | Riverine | Several |
| 4 | Peechelba | Aug. 1953 | Riverine | Several |
| 5 | Tallangatta | Aug. 1953 | Riverine | Several |
| 6 | Mildura | July 1953 | Riverine | Several |
| 7 | Woolpooer | Mar. 1954 | Non-riverine | Several |
| 8 | You Yangs | May 1954 | Savannah woodland | Several |
| 9 | Edenhope | May 1954 | Non-riverine | Two |
| 10–13 | Skipton | June 1954 | Savannah woodland | One |
| 14–19 | Mildura | Aug. 1954 | Riverine | One |
| 20–27 | Barmah | Aug. 1954 | Riverine | One |
| 28–31 | You Yangs | Sept. 1954 | Savannah woodland | All from one tree and from the N., S., E., and W. sides respectively. |

(c) *The Germination Dish*

The steps in dish preparation were (Fig. 2):

- (1) Water was poured into a 4-in. Petri dish to a depth of c. $\frac{1}{10}$ in.
- (2) As trip of gauze, 11 in. by 3 in., was moistened on a table and rolled to form a cylinder approximately 11 in. long by $\frac{1}{8}$ in. diameter which was then placed in the water around the perimeter of the Petri dish.
- (3) Two 11-cm filter papers were placed together and five equally spaced radial cuts were made extending almost to the centre. The filter papers were laid on the convex face of a watch glass of 3½ in. diameter, moistened, and smoothed out. The overlapping portions of the filter papers were then folded under the edge of the watch glass.
- (4) The watch glass was placed in the Petri dish, convex side up, so that its perimeter rested on the gauze wick.
- (5) Four pieces of green P.V.C. tubing, and one of red, were arranged radially to divide the convex surface into five equal sectors, thus permitting the germination of five subsamples in each dish. Red tubing was used as the marker, and the subsamples numbered from this in a clockwise direction.

It was necessary to replenish the supply of water after 7 days when working at temperatures above 85°F. It was found advantageous to use a small syringe for this purpose.

(d) *Selection of Seed Samples*

Both fertile seeds and chaff of *E. camaldulensis* are yellow-brown in colour. The dimensions of fertile seeds and chaff are as follows:

Seeds : 0.04–0.07 in. long, 0.02–0.04 in. diameter.

Chaff : 0.03–0.07 in. long, 0.02–0.03 in. diameter.

Some fertile seeds are bigger than the chaff and are of a characteristic shape, but the majority of the fertile seeds are not easily distinguishable from the chaff when dry. When imbibed, the white embryo of a fertile seed shows clearly through the seed coat, so that with a low-power binocular microscope one may confidently distinguish seeds from chaff.

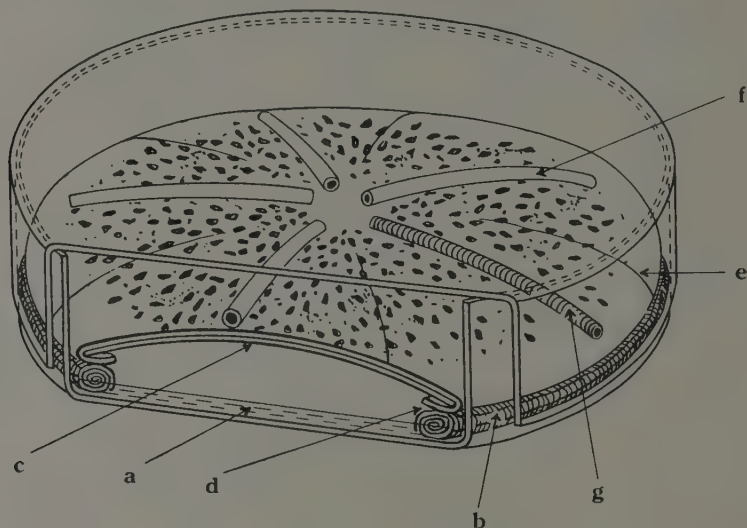


Fig. 2.—The germination dish. *a*, reservoir of water; *b*, gauze wick; *c*, watch-glass covered by two filter papers; *d*, filter paper edges folded under watch-glass; *e*, radial cut in filter paper; *f*, green P.V.C. tubing; *g*, red P.V.C. tubing.

Because of the smallness of the seeds and the difficulty in identifying fertile seeds in the dry state, weighed rather than counted samples were used.

In sampling, the seed-lot was poured onto a smooth table and mixed systematically using a wide flat spatula. This gave better randomization and proportional sampling than shaking in a glass jar, which tends to concentrate fertile seeds in certain parts of the seed-lot. After mixing, the seed was shaped into a disk, halved, and then quartered. Two opposite quarters were then selected and again mixed, halved, and quartered, and the procedure repeated until each quarter contained between 0.5 g and 1.0 g of seed. One such quarter was then selected and used to provide ten weighed subsamples for testing.

Because the number of fertile seeds per gram varies widely in different seed-lots it was found necessary to use subsamples varying in weight between 0.025 g and 0.075 g. By this means it was possible to arrange that each subsample contained between 30 and 50 fertile seeds.

TABLE 2
TYPICAL GERMINATION TEST RESULT SHEET

| Experiment No. R 1 | | | | | | Dish No. 1/95 | | | |
|---|--------------|-------|-------|-------|-------|--|--------|------------------------|--------------------|
| Species— <i>E. camaldulensis</i> | | | | | | Seed-lot No. 1 | | | |
| Treatment—Standard Series | | | | | | | | | |
| Germination temperature—95°F | | | | | | Weight of Samples—0.05 g | | | |
| Time of starting—0900 hours, 10. ii. 54 | | | | | | Time of finishing—1000 hours, 15. ii. 54 | | | |
| Date | 11.ii | 12.ii | 13.ii | 14.ii | 15.ii | Total Number of Germinations | Squash | Total Fertile Seeds | Germination (%) |
| Hours | 24 | 48 | 72 | 96 | 121 | | | | |
| Temperature (°F) | 94 | 95 | 95 | 94 | 95 | | | | |
| Subsample No. | Germinations | | | | | | | | |
| 1 | 3 | 14 | 11 | — | — | 28 | — | 28 | 100 |
| 2 | 2 | 19 | 13 | 1 | — | 35 | — | 35 | 100 |
| 3 | 4 | 21 | 11 | 1 | — | 37 | — | 37 | 100 |
| 4 | 3 | 20 | 14 | 1 | — | 39 | — | 39 | 100 |
| 5 | 4 | 20 | 6 | 1 | — | 31 | — | 31 | 100 |
| 6 | — | 27 | 11 | 2 | 1 | 41 | — | 41 | 100 |
| 7 | 4 | 15 | 6 | 1 | 1 | 27 | 1 | 28 | 96.4 |
| 8 | 6 | 14 | 8 | — | — | 28 | 1 | 29 | 96.5 |
| 9 | 4 | 25 | 7 | 1 | — | 37 | — | 37 | 100 |
| 10 | 4 | 25 | 15 | — | — | 44 | — | 44 | 100 |
| Total | 34 | 200 | 102 | 8 | 3 | 347 | 2 | 349 | 99.3 (mean) |
| Progressive Total | 34 | 234 | 336 | 344 | 347 | | | | |
| Progressive percentage | 9.7 | 67.0 | 96.2 | 98.6 | 99.4 | | | | |
| Number of fertile seeds per gram of sample | | | | | | 698 | | | |
| Number of seeds germinated per gram of sample | | | | | | 694 | | | |
| Germinative Energy Index = 9.7 + 67.0 + 96.2 + 98.6 | | | | | | = 271 | | | |

(e) *Checking Germination; Expression and Analysis of Results*

Seeds were recognized to have germinated when the hypocotyl had emerged and grown to approximately $\frac{1}{8}$ in. in length. Germinated seeds were counted and removed daily in all treatments except those requiring continuous light or darkness. The data for germination for each subsample were recorded separately (Table 2).

As the seed-lots were under test together in each treatment, the length of the germination period for all lots was dependent on the seed-lot which germinated most

slowly. The end of the germination period for a given treatment was determined as the day when the total percentage germination for the ten subsamples of all lots fell to less than 1 per cent. for treatments which gave high, and to nil for treatments which gave low percentage germination.

At the end of the germination period, all particles remaining on the filter paper were systematically squashed using a narrow spatula. Those which when squashed showed the presence of a firm white embryo were classified as "viable". The number of fertile seeds in each subsample was represented by the number which germinated plus the number of ungerminated viable seeds.

The mean of the percentage germination of each of the ten subsamples was taken as the final percentage germination for the seed-lot in the treatment. However, the duration of germination varied between treatments, ranging from a few days under optimum or near optimum conditions to over 20 days (see Table 3). Consequently, a direct comparison of the responses by different seed-lots to various treatments based only on final percentage germination gives no indication of variation in germinative energy.

Germinative energy was expressed by an index (G.E.I.) derived by summing the progressive total percentage germination for the first 4 days of the germination period (see example in Table 2).

This index in effect gives an easily calculable numerical indication of the area under the progressive curve of percentage germination against time for a germination period of 4 days. A period of 4 days was taken to provide numerical comparisons between energies of most seed-lots in most treatments. There were few treatments in which no germination was recorded by the fourth day. A high index denotes early and rapid germination, and a low one late and slow germination.

In statistical analysis of the data the percentage germination of each subsample was converted to degrees using the appropriate table in Fisher and Yates (1953). The means of the ten transformed values for each seed-lot were used in a paired *t* test to compare effects of different treatments on a set of seed-lots. A *t* test using the pooled standard deviation was carried out to compare responses of two seed-lots in the same treatment and to compare responses of the same seed-lot in different treatments. Appropriate *t* tests were also used to test for differences in germinative energy.

It may be seen from Table 2 that the number of fertile seeds varied somewhat between subsamples. Had the results of comparisons between treatments been of doubtful statistical significance this variation between subsamples could have been embarrassing and would have necessitated a more elaborate statistical analysis taking into account this variation. However, the conclusions reached are based on highly significant comparisons and it is felt that further investigation of subsample variability is unnecessary.

It should be noted that for high percentage germination the distribution of percentages is not statistically normal.

(f) *Temperature and Light Control*

(i) *Temperature*.—Treatments requiring temperatures of 65°F and above were carried out using incubators where the differential was approximately $\pm 1^\circ\text{F}$.

Constant-temperature equipment used to give temperatures below 65°F had a differential of $\pm 2^\circ\text{F}$. In the instance of alternating temperature work, two constant-temperature units were used, the dishes being transferred at the scheduled time from one to the other.

TABLE 3

FINAL PERCENTAGE GERMINATION, GERMINATIVE ENERGY INDEX, AND GERMINATION PERIODS FOR ALL SEED-LOTS AND TEMPERATURES USED IN THE STANDARD SERIES

| Temperature of Treatment (°F) | No. of Seed-lots Treated | Final Germination (%) | | Energy Index | | Germination Period (hours) |
|-------------------------------|--------------------------|-----------------------|------|--------------|------|----------------------------|
| | | Mean | S.D. | Mean | S.D. | |
| 110 | 6 | 0 | — | — | — | 220 |
| 108 | 6 | 29 | 15.9 | 14 | 6 | 220 |
| 105 | 6 | 82 | 10.9 | 90 | 48 | 220 |
| 100 | 11 | 92 | 4.4 | 236 | 35 | 144 |
| 100* | 6 | 89 | 2.9 | 224 | 29 | 144 |
| 95 | 31 | 98 | 2.8 | 262 | 39 | 120 |
| 95* | 6 | 96 | 2.3 | 261 | 38 | 120 |
| 90 | 11 | 87 | 7.4 | 130 | 83 | 192 |
| 90* | 6 | 88 | 8.2 | 137 | 81 | 192 |
| 80 | 11 | 64 | 20.9 | 88 | 64 | 192 |
| 70 | 7 | 15 | 13.3 | 25 | 29 | 192 |
| 70* | 6 | 13 | 10.8 | 21 | 27 | 192 |
| 52 | 6 | 26 | 23.4 | 0 | — | 600 |
| 47 | 8 | 0 | — | — | — | 600 |

*Repeat runs.

(ii) *Light*.—A 15-watt filament lamp was used in the 80, 85, and 95°F continuous light and alternating light-dark treatments. Two 40-watt warm-white fluorescent tubes, together with diffuse daylight, were the light sources for all other light treatments.

The approximate intensities at the dishes were:

| | | | | | |
|--------------------------------|----|----|----|----|------------------|
| 15-watt filament lamp | .. | .. | .. | .. | 11 foot-candles |
| 80-watt fluorescent + daylight | .. | .. | .. | .. | 125 foot-candles |
| 80-watt fluorescent | .. | .. | .. | .. | 121 foot-candles |

The dishes were arranged on a flat tray to expose the subsamples to the light as uniformly as possible.

III. EXPERIMENTAL

(a) *Influence of Temperature on Germination*

(i) *Constant Temperature Standard Series*.—The term “standard” signifies that the seed samples were germinated in darkness except for approximately 10 min exposure to light each day when the dishes were checked for germination.

The constant temperatures used in this series ranged from 47 to 110°F. The actual temperatures and the results obtained are shown in Table 3 and Figures 3–5.

The mean percentage germination and mean germinative energy increased with temperature from 70°F to a maximum at 95°F and then decreased to nil at 110°F. The higher mean percentage germination at 52°F than at 70°F is discussed later.

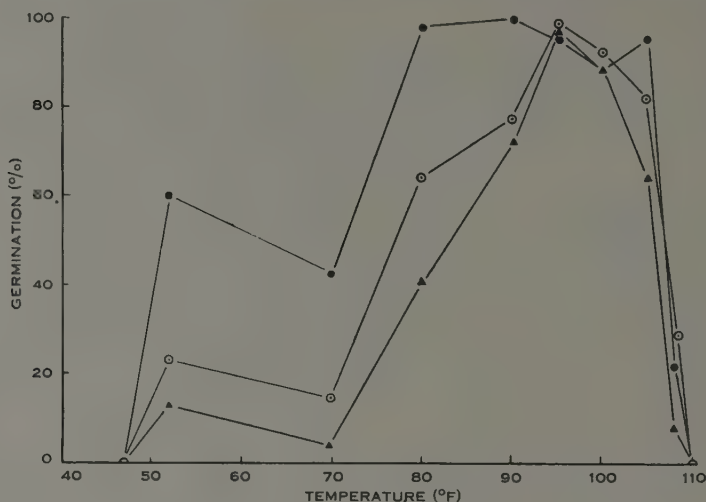


Fig. 3.—Percentage germination of *E. camaldulensis* seeds at different constant temperatures. ○, mean germination of seed-lots 1-6; ●, germination of seed-lot 6; ▲, germination of seed-lot 3.

After 220 hours at 110°F the fertile seeds had darkened, mould growth was heavy and the possibility of germination seemed remote. When removed from the incubator, five subsamples from each seed-lot of the seeds used in the 110°F treatment were

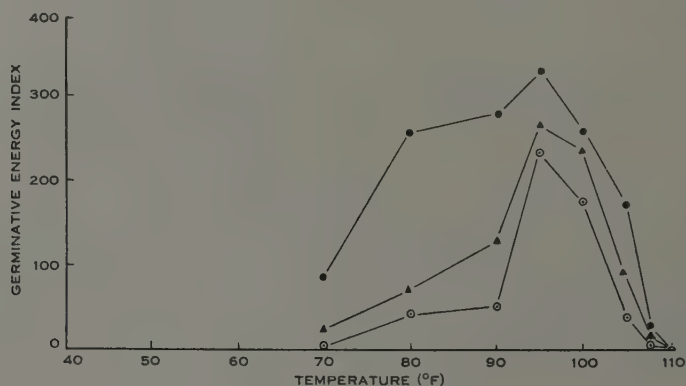


Fig. 4.—Effect of constant temperature on germinative energy of *E. camaldulensis* seeds. ▲, mean germinative energy indices of seed-lots 1-6; ●, G.E.I. of lot 6; ○, G.E.I. of lot 3.

transferred to 70°F and five to 100°F. After 144 hours the mean percentage germination was 3.4 per cent. at 70°F and 5.3 per cent. at 100°F. The squashed embryos of most of the remaining originally viable seeds were yellow-brown and soft. This

indicated that storage at 110°F for 220 hours is lethal to most imbibed *E. camaldulensis* seeds. Very few seeds were killed by treatment at 105°F but treatment at 108°F caused considerable mortality.

Each of the 31 seed-lots gave rapid and nearly complete germination at 95°F while slower, lower, and more variable responses resulted at higher and lower temperatures (Table 3). This indicated that the most suitable constant temperature for germination of *E. camaldulensis* seeds under the described conditions of moisture and light lies close to 95°F irrespective of provenance.

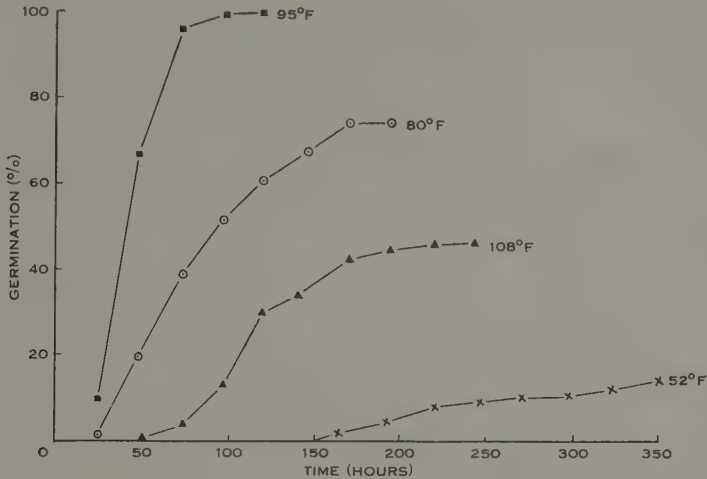


Fig. 5.—Typical graphs of progressive percentage germination for *E. camaldulensis* seeds at constant temperature (seed-lot 2).

The data for germinative energy for seed-lots 1–6 at all consecutive pairs of temperatures were significantly different (1 per cent. level). The data for percentage germination for these seed-lots at consecutive pairs of temperatures, except 52 and 70°F, 90 and 95°F, and 100 and 105°F also showed differences significant at the 1 per cent. level of probability. The seed-lots were then compared individually in these three pairs of temperatures, and showed the following differences:

52 and 70°F.—The mean final percentage germination for four lots at 52°F was significantly higher (1 per cent. level) than at 70°F whereas lots 3 and 5 were significantly higher (1 per cent. level) at 70°F than at 52°F.

90 and 95°F.—The mean final percentage germination for four lots at 95°F was significantly higher than at 90°F (two at the 1 per cent. and two at the 5 per cent. level). There was no difference at these temperatures with one lot, and one was significantly better (1 per cent. level) at 90°F than at 95°F.

100 and 105°F.—The mean final percentage germination for four lots at 100°F was significantly higher than at 105°F (three at the 1 per cent. and one at the 5 per cent. level). There was no difference at these temperatures with one lot, while one was significantly better (5 per cent. level) at 105°F than at 100°F.

(ii) *Effect of Stratification on Germination*.—The effect of stratification on subsequent germination at a higher temperature was studied. Samples of imbibed seeds from six seed-lots were stored at 47°F for 600 hours and then transferred to 95 and 70°F. Germination was extremely rapid at 95°F, averaging 91 per cent. at the end of the first 24 hours of the germination period and 100 per cent. after 96 hours. Germination at 70°F was rapid for the first 48 hours of the germination period but few seeds germinated thereafter. In Figure 6, the data for mean percentage germination of the six seed-lots used in these two treatments are compared with the data for the "standard" tests on the same six seed-lots. When germination ceased at 70°F the stratified seed samples were transferred to 95°F. The percentage germination rapidly rose to 100 per cent. for all lots within 50 hours (see Fig. 6).

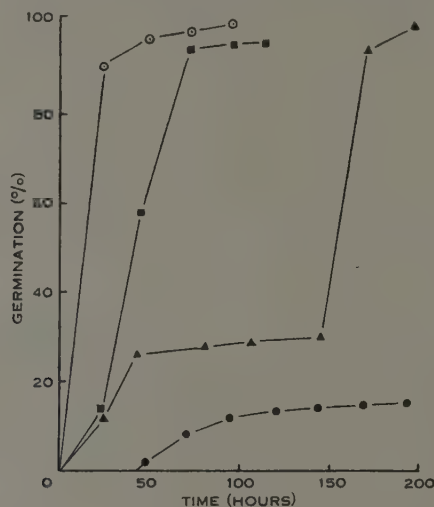


Fig. 6.—Effect of moist storage at 47°F for 600 hours in germination of *E. camaldulensis* seeds. ○, transferred to 95°F; ▲, transferred to 70°F and later to 95°F. ■, germination at 95°F without pretreatment; ●, germination at 70°F without pretreatment. (Means of seed-lots 1-6.)

The mean final percentage germination and mean germinative energy indices for the two stratification treatments were 100 per cent. and 387 for 95°F, and 29 per cent. (S.D. 18.3) and 84 for 70°F. All except the final percentage germination at 95°F were significantly different from results for the corresponding temperatures in the standard series. This may be due to the effect of stratification in eliminating the requirement for light in germination (see Section III(c)). Black and Wareing (1955) showed that stratification markedly decreased the requirement for light in the germination of the light-demanding seeds of *Betula pubescens*. The apparent stimulating effect of stratification may be due in part to the fact that the seeds were fully imbibed when transferred to 70 and 95°F, whereas in the standard series the germination period was taken from the moment the dry seeds were shaken onto the moist substratum.

The high final percentage germination obtained at 52°F in the standard series (see Table 3) may also be associated with this effect of cold moist storage on light requirements for germination. In this treatment lots 1 and 6 gave relatively high mean final percentage germination (47 and 58 per cent. respectively), compared with 14 and 43 per cent. at 70°F in the "standard" series. The percentage germination at 52°F was significantly higher (1 per cent. level) than at 70°F for both seed-lots. Later these two proved to be the least light-demanding of the six seed-lots, and would then possibly be the first to show the effect of stratification.

(iii) *Effect of a Short Period at Near Optimum Temperature on Subsequent Germination at Infra-optimal Temperatures.*—Seed samples of seven lots were allowed to germinate for 192 hours at 70°F. The mean percentage germination was 15 per cent. (S.D. 13.3), and germination appeared to have finished. The samples were then

TABLE 4
GERMINATION RESULTS FROM THE BOOSTING SERIES

| Boosting Period at 95°F (hours) | Means and Standard Deviations of Germination Percentage (8 Lots) | | | | | |
|--|--|------|------|------|------|------|
| | 52°F | | 70°F | | 95°F | |
| | Mean | S.D. | Mean | S.D. | Mean | S.D. |
| 8 | 11** | 14.4 | 33* | 18.4 | 99.7 | 0.8 |
| 16 | 31** | 21.2 | 42 | 25.8 | 99.6 | 0.7 |
| 8 | | | +16* | 15.1 | 98.3 | 1.5 |
| 16 | | | +36 | 29.6 | 98.5 | 2.1 |

* and + Significantly different at the 5 per cent. level of probability.

** Significantly different at the 1 per cent. level of probability.

kept for 4 hours at 100°F and replaced in the 70°F incubator. Within a short time sufficient seeds had germinated to raise the percentage germination to 73 per cent. (S.D. 24.6). After this initial burst of germination few or no seeds germinated; 120 hours after the first high temperature treatment the remaining seeds were given another similar treatment and returned to 70°F. During the next 72 hours another burst of germination occurred bringing the percentage germination up to 83 per cent. (S.D. 11.8). These responses demonstrated that only a short period of near optimum temperature was required to stimulate the germination of a large percentage of imbibed *E. camaldulensis* seeds at an infra-optimal temperature.

This effect was further instanced in a "boosting" series of four trials. Here the seeds were treated for periods of 8 hours and 16 hours at 95°F before germination at 52 or at 70°F. The seeds had to imbibe during the period at 95°F. When germination was considered complete in the two 52°F trials, the remaining seeds were transferred to 70°F until the rate of germination again fell off, then the residual samples were transferred to 95°F for a further period. Similarly, the residual samples from the two 70°F trials were transferred to 95°F for a second germination period. The means and

standard deviations of the final percentage germination resulting from these treatments are shown in Table 4.

The only apparent effect of the 8-hours "boost" was an earlier start of germination than in the 52 and 70°F treatments of the standard series. There was no significant increase in percentage germination at either temperature; in fact it was significantly lower (5 per cent. level) at 52°F in the boost trials than in the 52°F standard series. This low response may be attributed to the early start of germination and the shorter germination period of 260 hours. In the 52°F standard series trial, germination did not start until after 150 hours and the germination period was 600 hours. Hence the seeds in the 52°F boosting treatment were not given time to show the effect of cool moist storage which is thought to have been responsible for the late and high germination in the 52°F standard series treatment. It is possible that, had the seeds been left for 600 hours, a number of them might have germinated between 350 and 550 hours as in the standard series treatments.

TABLE 5

MEAN PERCENTAGES GERMINATION AND ENERGY INDICES FOR THE ALTERNATING TEMPERATURE SERIES

| Treatment No. | Temperature Alternation (°F) | Periods of Alternation (hours) | Germination (%) | | Energy Index | | Germination Period (hours) |
|---------------|------------------------------|--------------------------------|-----------------|------|-----------------|------|----------------------------|
| | | | Mean for 6 Lots | S.D. | Mean for 6 Lots | S.D. | |
| 1 | 105-70 | 24-24 | 91 | 11.7 | 80 | 55 | 192 |
| 2 | 100-70 | 24-24 | 97 | 2.9 | 140 | 70 | 192 |
| 3 | 95-70 | 24-24 | 95 | 4.1 | 184 | 66 | 168 |
| 4 | 95-70 | 8-16 | 98 | 2.4 | 218 | 20 | 168 |
| 5 | 95-70 | 6-18 | 93 | 6.7 | 158 | 54 | 168 |
| 6 | 95-70 | 4-20 | 88 | 8.6 | 158 | 54 | 168 |
| 7 | 95-52 | 8-16 | 90 | 5.3 | 146 | 40 | 168 |

The high response shown to 4 hours at 100°F preceded and followed by germination at 70°F and the negligible effect on germination capacity at 70°F caused by 8 hours of pretreatment at 95°F appear anomalous. A partial explanation may be that the seeds in the former case were fully imbibed when they received the high temperature treatment. Seeds in the latter case were dry at the start, and it is doubtful whether these were fully imbibed by the end of the period of 8 hours at 95°F. The period of moist storage at infra-optimal temperature in the former treatment may have also contributed to the high response.

The effect of cool moist storage was manifested in the higher percentage germination at 70°F after a period at 52°F compared with those seed samples which, after treatment at 95°F, were transferred to 70°F. This effect is illustrated by comparing the percentage germination at 70°F for the two 8-hour boosts (Table 4).

The 16-hour boosting periods had a significant effect on both germinative energy and capacity. An initial treatment for 16 hours at 95°F followed by a period at 52°F

did not result in a greater percentage germination than continuous treatment at 52°F but a similar initial treatment followed by treatment at 70°F gave a significantly greater percentage germination than continuous treatment at 70°F.

(iv) *Alternating Temperature Series*.—This series comprised seven trials involving combinations of different temperatures and periods (see Table 5).

The period of light given (c. 10 min each day when checking for germination) was similar to that in the standard series. Seed-lots 1-6 were used in these treatments to assess the likelihood of improving on the levels of germinative capacity and energy obtained in the 95°F standard series treatment, and to determine whether the germination of seeds collected in cooler climates is promoted by shorter periods of warm day temperatures or lower night temperatures than that of seeds collected in warmer climates. The results are given in Table 5.

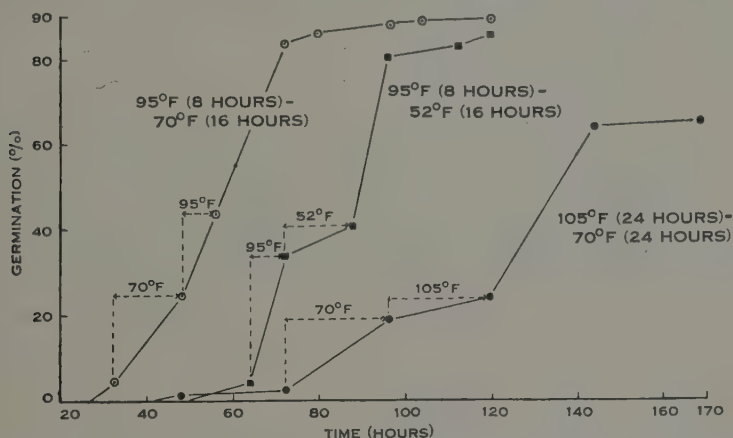


Fig. 7.—Typical germination of *E. camaldulensis* seeds in alternating temperatures (lot 5).

A daily alternation of 95°F for 8 hours with 70°F for 16 hours gave significantly higher germinative energy than the other six treatments. Mean percentage germination in this treatment was greater than in the other treatments of the series but it was significantly higher than the mean percentage germination in treatments 6 and 7 only.

Mean percentage germination in the 95°F treatment of the standard series was as high and the germinative energy significantly higher (1 per cent. level) than in the 95-70°F alternating temperature treatment. It seemed apparent then, from the results of the seven treatments of the series, that alternating temperatures would not result in more rapid or complete germination than a constant temperature of 95°F, hence no further combinations were tried.

A smooth progressive germination graph was obtained only with treatments 3 and 4, whereas in treatments 1 and 2 the mean per hour germination percentages were greatest in the 70°F period, and in treatments 5 and 7 in the 95°F periods (see Fig. 7).

There was no apparent relationship between germinative energy or capacity and climate of the locality of collection in the treatments tried in this series.

(b) *Effect of Light on Germination at Constant Temperature*

Clifford (1953), in his preliminary studies on light requirements for germination of seeds of some species of *Eucalyptus*, reported that immature seeds of *E. camaldulensis* required light, but gave no detailed information on this or the light requirements of mature seeds.

The treatments below were designed to ascertain the effects of different periods of light on the germination of *E. camaldulensis* seeds at a constant temperature of 80°F. The treatments were as follows:

- (1) Continuous light.
- (2) Continuous light broken by a single 2-hour period of darkness at 144 hours, i.e. continuous light until germination ceased, then 2 hours of darkness followed by continuous light.
- (3) Continuous darkness.
- (4) Continuous darkness broken by short periods of light. In this treatment the extent of germination in darkness was first ascertained. The remaining seeds were then given 2 min of light and returned to darkness until germination again ceased, so giving a characteristic response to 2 min of light for the seed-lots tested. Seeds remaining after this second dark period were given further periods of 10, 30, and 28 minutes of light with intervening periods of darkness.
- (5) Daily alternation of an 8-hour period of light with a 16-hour period of darkness.
- (6) A daily period of 10 min of light.

The types and intensities of light used were indicated in Section II(f).

Eight seed-lots were used in the first set of six treatments. Later, these same seed-lots together with another 17 lots were used in three of the treatments to provide further data and to act as a check on the previous results (see Table 6).

In the continuous light and continuous darkness treatments, the extent of germination was not checked daily to avoid interference by altered light conditions. Dark germination was regarded as complete at the end of a germination period estimated on the basis of earlier experiments at 80°F. These estimates proved successful because no early stages of germination were found when the dishes were checked, indicating that germination had finished 1—2 days earlier. Seeds in continuous light were easily inspected while in the incubators, and when no newly germinated seeds were observed the dishes were removed and the germinated seeds counted. Germination in the other treatments was checked and counted daily, and was regarded as complete when no increase in the percentage germination was observed over a period of 24 hours.

The results are given in Table 6. The mean percentage germination in continuous light was approximately 14 to 15 times that in continuous darkness, indicating that light markedly stimulates the germination of moist seeds at 80°F. A period of light

as short as 2 min following germination in continuous darkness increased the mean percentage germination in darkness for 8 seed-lots from 4.3 to 18.4 per cent. Further short periods of light periodically interrupting the darkness stimulated germination until the mean percentage germination reached 81.3 per cent. (see Figs. 8 and 9).

Although the percentage germination in light was high considering that the temperature was approximately 15°F below optimum, germination of some seeds may have been inhibited by continuous light. This effect was indicated by the rapid and significant increase (5 per cent. level) in percentage germination from 69.1 to 78.0 per cent. in response to interruption of the continuous light by a 2-hour period of darkness when germination in light had ceased.

TABLE 6
MEAN PERCENTAGE GERMINATION FOR THE LIGHT TREATMENTS AT 80°F

| Treatment | | No. of Seed-lots Treated | Percentage Germination | |
|-----------------|---------------------------|--------------------------------|------------------------|------|
| No. | Description | | Mean | S.D. |
| 1 | Continuous light | 8 | 69.1* | 17.3 |
| †1 | Continuous light | 25 | 74.4 | 19.1 |
| 2 | 2-hour period of darkness | 8 | 78.0* | 11.1 |
| 3 | Continuous darkness | 8 | 4.3 | 2.9 |
| †3 | Continuous darkness | 25 | 5.1 | 3.1 |
| 4 ^a | 2 min of light | 8 | 18.4 | 14.1 |
| 4 ^b | 2+10 min of light | 8 | 44.1 | 21.7 |
| 4 ^c | 2+10+30 min of light | 8 | 68.6 | 18.8 |
| 4 ^d | 2+10+30+28 min of light | 8 | 81.3** | 16.0 |
| †4 ^a | 2 min of light | 25 | 24.1 | 16.3 |
| †4 ^b | 2+10 min of light | 25 | 46.7 | 20.2 |
| 5 | 8-16 hour light-dark | 8 | 77.5 | 17.1 |
| 6 | 10 min of light each day | 8 | 66.8** | 18.1 |

* Significantly different at the 5 per cent. level of probability.

** Significantly different at the 1 per cent. level of probability.

† Repeat runs using 25 seed-lots.

It could be argued on the basis of the results that a total of 70 min of light given as 10 min each day of the germination period was not as effective as a similar quantity given in periodic increments of 2, 10, 30, and 28 min, i.e. 66.8 per cent. compared with 81.3 per cent. mean percentage germination respectively. This may indicate that the length of the continuous light period is more important than the total duration of light. However, germination periods for the two trials were 192 hours and 624 hours respectively, and this may account for some of the difference.

Taking into account both germinative energy and capacity, the best response was shown by the 8 hour-16 hour light-dark alternation, this being better than 10 min of light daily. Mean energy indices and percentage germination here were 172 and 77.5 per cent. for 8 hours of light daily, and 74 and 66.8 per cent. for 10 minutes of light each day. The energy indices were significantly different at the 1 per cent. level, and

the percentage germination at the 5 per cent. level of probability. Continuous light resulted in a lower mean percentage germination than 8 hours of light per day. No

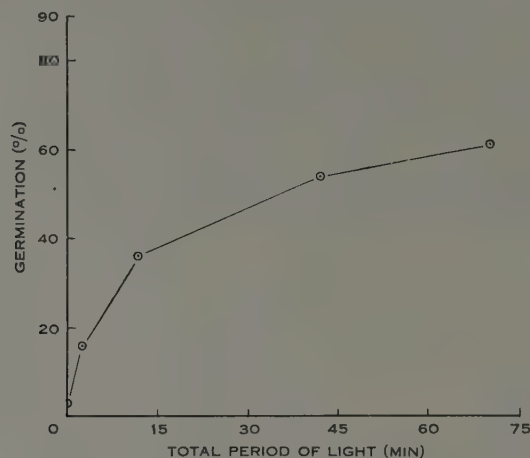


Fig. 8.—Germination responses to brief periods of light given during germination in darkness at 80°F (means of 8 seed-lots).

germinative energy comparison was available here. The better response to 8 hours of light per day compared with 10 min of light per day was much more marked for some

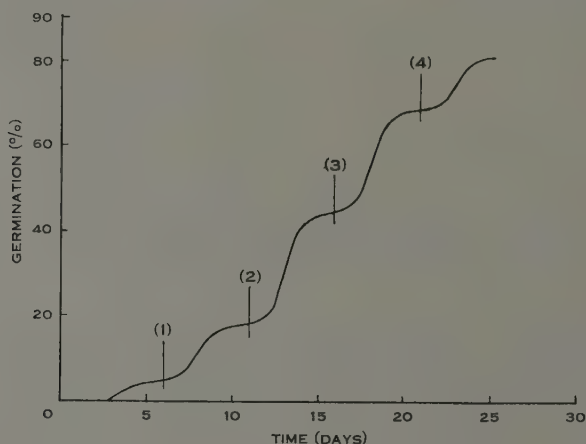


Fig. 9.—Graphic representation of germination response to short periods of light during germination in darkness at 80°F (means of 8 seed-lots). Seeds were given (1) 2 min, (2) 10 min, (3) 30 min, and (4) 28 min of light after 6, 11, 16, and 21 days respectively in darkness.

seed-lots than for others (see Table 7). The magnitude of this variation may indicate variations between seed-lots in light requirements for germination, i.e. a large difference in response indicates a high light requirement, and vice versa.

Variation in the light requirements between lots of *E. camaldulensis* seeds does not seem to bear any relationship to maturity of the seed sample. Included in the 25 seed-lots tested were seeds extracted from very ripe and others from somewhat green capsules. Different lots of relatively immature seeds were found to have either a high or a low light requirement. A similar variation existed between mature seed-lots.

The variation in light requirements demonstrated by the results listed in Tables 6 and 7 is, however, confused with varying responses by the seed-lots to infra-optimal temperature. When light is not limiting, different seed-lots vary in their capacity to germinate at temperatures below the optimum. This is somewhat clarified in the experiments outlined below where the interaction of light and temperature has been studied.

TABLE 7
PERCENTAGE GERMINATION AND ENERGY INDICES FOR THE 10-MINUTE AND 8-HOUR DAILY LIGHT TREATMENTS AT 80°F

| Seed Lot No. | 10 Minutes of Light Daily | | 8 Hours of Light Daily | |
|--------------|---------------------------|--------------|------------------------|--------------|
| | Percentage Germination | Energy Index | Percentage Germination | Energy Index |
| 1 | 63 | 92 | 73 | 183 |
| 2 | 74 | 112 | 75 | 163 |
| 3 | 41 | 43 | 69 | 120 |
| 4 | 75 | 102 | 86 | 179 |
| 5 | 70 | 91 | 86 | 172 |
| 6 | 97 | 256 | 98 | 285 |

(c) *Interaction of Light and Temperature*

Earlier trials had indicated that light became less limiting as the temperature approached 95°F. The relationship between light and temperature was studied by testing the germination of eight lots of seeds in continuous light and continuous darkness at each of 70, 80, 85, and 95°F.

Seed samples from the continuous darkness series were given a short period of light after dark germination was complete and were then replaced in the dark to obtain information on the effect of short periods of light at different temperatures.

Further tests were carried out using 13 seed-lots subjected to 8 hours of light daily at constant temperatures of 70, 85, and 95°F.

(i) *Continuous Light Series*.—Data for mean percentage germination for eight lots used at the four temperatures of this series are given in Table 8.

Mean percentage germination at each infra-optimal temperature in this series was higher than at corresponding temperatures in the standard series, although the 95°F results were not significantly different. This higher percentage germination was more marked at temperatures below optimum than close to it, suggesting that light has a greater effect at infra-optimal temperatures. It appears then that temperature

becomes less important in germination as light conditions approach their optimum, and vice versa. This effect is perhaps better illustrated by comparison of the data above with those for continuous darkness at different temperatures (see (ii) below).

TABLE 8
MEAN PERCENTAGE GERMINATION OF EIGHT SEED-LOTS TREATED IN
CONTINUOUS LIGHT

| Germination Temperature (°F) | Percentage Germination | |
|------------------------------|------------------------|------|
| | Mean | S.D. |
| 70 | 27.3 | 17.6 |
| 80 | 74.4 | 19.1 |
| 85 | 86.9 | 8.7 |
| 95 | 99.9 | 0.4 |

It should also be noted here that continuous light at 95°F did not appear to restrict germination of some seeds as seemed to be the case at 80°F. A study of the germinative energy of seeds germinating in continuous light at 95°F may indicate a retardation compared with seeds germinated at 95°F with only 8 hours of light daily.

TABLE 9
MEAN PERCENTAGE GERMINATION OF EIGHT SEED-LOTS IN CONTINUOUS
DARKNESS AT FOUR CONSTANT TEMPERATURES

| Germination Temperature (°F) | Percentage Germination | |
|------------------------------|------------------------|------|
| | Mean | S.D. |
| 70 | 2.1 | 1.2 |
| 80 | 6.9 | 3.6 |
| 85 | 8.4 | 6.7 |
| 95 | 61.3 | 22.1 |

(ii) *Continuous Darkness Series*.—The results in this series (see Table 9), taken in conjunction with data given above, strongly indicate the necessity of light for germination at temperatures below 95°F. Variation between seed-lots at 95°F was wide, the mean percentage germination for lots 1, 2, 5, and 6 being 74, 55, 37, and 96 per cent. respectively. It should be noted here that lots 1 and 6 which responded least to light, also had the highest germination capacities at 52°F in the standard series. It has already been suggested that stratification at 52°F partially annulled the requirement for light. Seed-lots with low light requirements should show this effect to a greater extent than lots with a high light requirement.

When germination in continuous darkness at 95°F had ceased, the remaining seeds were exposed to light for 3 min. Nearly all the remaining seeds then germinated rapidly. The mean percentage germination by the end of the second dark period was 97.6 per cent (S.D. 5.3). The percentage germination of lot 5 was increased from 37 to 100 per cent. by exposure to light for 3 min, whereas the percentage germination of lot 3, which appeared to require more light than other seed-lots studied, was raised from 40 to 85 per cent. A second short period of light given to the remaining seeds resulted in 100 per cent. germination of all seed-lots after a further short time in the dark.

TABLE 10

MEAN PERCENTAGE GERMINATION AND GERMINATIVE ENERGY OF 13 SEED-LOTS GERMINATED AT CONSTANT TEMPERATURE AND GIVEN 8 HOURS OF LIGHT DAILY

| Temperature (°F) | Percentage Germination | | Energy Index | |
|------------------|------------------------|------|--------------|------|
| | Mean | S.D. | Mean | S.D. |
| 95 | 100 | — | 278 | 18 |
| 85 | 86.1 | 13.4 | 205 | 49 |
| 80 | 77.5 | 17.1 | 172 | 65 |
| 80 | 79.8 | 15.3 | 167 | 63 |
| 80 | 78.6 | 16.7 | 168 | 61 |
| 70 | 40.6 | 26.6 | 42 | 43 |

Five minutes of light given to the seed samples after the initial period in darkness at 70°F increased the mean percentage germination from 2.1 to 28.6 per cent. (S.D. 14.9). A second period of 8 min of light resulted in a mean percentage germination of 49.8 per cent. (S.D. 18.7). Similar light treatments given to the residual seeds at 85°F increased the mean percentage germination from 8.4 to 57.7 per cent. (S.D. 20.9) and then to 74.5 per cent. (S.D. 16.1). The effect of short periods of light following germination in the dark at 80°F has been described earlier.

(iii) *Interaction between Temperature and Daily Alternation of Light and Darkness.*

—Interesting data were obtained from experiments in which the seeds were treated at different temperatures with a daily alternation of 8 hours of light and 16 hours of darkness. The mean percentage germination and the mean germinative energy were proportional to the temperature up to 95°F when each of the 13 seed-lots germinated rapidly and completely. The results are given in Table 10. Three trials carried out at 80°F gave closely similar results, indicating that the sampling methods and germination techniques used are efficient and reasonably sound for treatment comparisons using statistical methods.

The germinative energy at 95°F was the highest obtained throughout the whole investigation of the effects of light and darkness.

(d) *Comparison of Four Light Conditions at Different Temperatures*

The data for mean percentage germination for 6 seed-lots, and mean germinative energy indices where available, are shown in Table 11 and Figure 10. No tests were carried out using 8 hours of light daily at 100°F.

TABLE 11
MEAN PERCENTAGE GERMINATION AND GERMINATIVE ENERGIES OF SIX SEED-LOTS IN FOUR DIFFERENT LIGHT TREATMENTS

| Treatment | 70°F | | | 80°F | | | 95°F | | | 100°F | | |
|------------------------|-----------------|------|--------|-----------------|------|--------|-----------------|------|--------|-----------------|------|--------|
| | Germination (%) | | Energy | Germination (%) | | Energy | Germination (%) | | Energy | Germination (%) | | Energy |
| | Mean | S.D. | Mean | Mean | S.D. | Mean | Mean | S.D. | Mean | Mean | S.D. | Mean |
| | Mean | S.D. | Mean | Mean | S.D. | Mean | Mean | S.D. | Mean | Mean | S.D. | Mean |
| Continuous light | 22 | 15.1 | 33* | +67 | 18.7 | 134** | 99.9 | 0.3 | 284* | 76 | 16.9 | |
| 8 hours of light daily | 28** | 16.6 | 22* | +81* | 16.3 | 63 | 100** | — | 21 | 89 | 2.9 | 224 |
| 10 min of light daily | 15** | 13.2 | | 70* | 17.6 | 42 | 96** | 2.3 | 38 | 26 | 7.9 | 29 |
| Continuous darkness | 2 | 1.4 | | 7 | 3.8 | | 58 | 20.7 | | | | |

* and + Within temperature differences significant at 5 per cent. level.

** Within temperature differences significant at 1 per cent. level.

Mean percentage germination and germinative energy obtained with 8 hours of light daily were significantly higher than those resulting from 10 min of light daily at 70, 80, and 95°F. The mean percentage germination in continuous light at 80°F was significantly lower than that produced by 8 hours of light daily at this temperature. There was no significant difference between these treatment pairs at 70 and 95°F.

Mean percentage germination in continuous darkness was significantly lower (1 per cent. level) at all temperatures than in the other light treatments. It was notable that the mean percentage germination in darkness at 100°F was significantly lower (1 per cent. level) than at 95°F. This difference, taken in conjunction with the low percentage germination in darkness at infra-optimal temperatures discussed earlier, indicates the vital role of light in germination at temperatures both above and below 95°F.

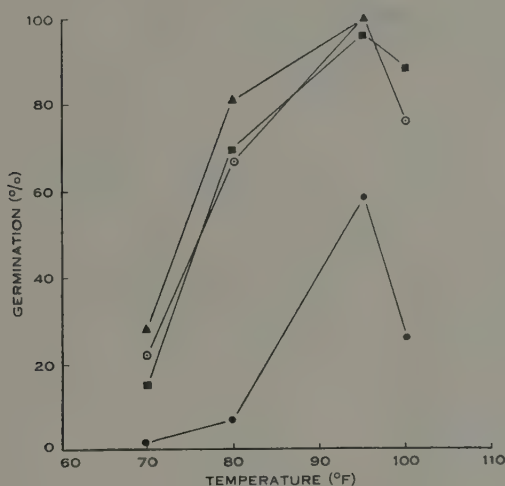


Fig. 10.—Comparison of mean percentage germination of six lots of *E. camaldulensis* seeds at different constant temperatures. ○, continuous light; ▲, 8 hours of light daily; ■, 10 min of light daily; ●, continuous darkness. (Lots 1-6.)

The mean percentage germination at 70, 80, and 85°F in continuous light was significantly greater than that at these temperatures in the standard series (see Table 3). However, a converse difference significant at the 5 per cent. level was obtained at 100°F, indicating an inhibitory effect of continuous light at this temperature. Further evidence of an inhibitory effect of continuous light was obtained in the trials with continuous light at 80°F but not at 95°F.

It appears then that certain amounts of light are required for satisfactory germination at all temperatures, but a daily dark period is also required for maximum germination at temperatures other than 95°F.

The rapid and complete germination at 95°F with 8 hours of light daily indicates that these conditions lie close to the optimum for the germination of *E. camaldulensis* seeds.

(e) Variation in Germination Response between Seed-lots

(i) *Lots Collected from Different Localities.*—In the early stages of this research it was thought that seeds collected from places with different climates might show some differences in their optimum requirements for germination. This aspect was first studied using seeds collected from trees along the Murray River from Mildura to Tallangatta, growing under a range of conditions of temperature and of rainfall. The germination responses to temperature and to light treatments of the individual lots of seeds were studied in relation to the following:

Maximum and minimum temperatures prevailing at the usual time of flood recession;

Maximum and minimum temperatures prevailing during the months of highest rainfall;

Variation in length of the daily warm period.

No correlation between germination requirements and any of these features of the climate was found.

TABLE 12

COMPARISON OF THE RANGE OF MEAN PERCENTAGE GERMINATION OF SIX RIVERINE SEED-LOTS WITH THOSE OF FOUR NON-RIVERINE LOTS

| Treatment | Range of Mean Percentage Germination | |
|-----------------------|--------------------------------------|---------------------|
| | 6 Riverine Lots | 4 Non-riverine Lots |
| 95°F standard series | 92-99 | 94-100 |
| 95°F continuous light | All 100 | All 100 |
| 80°F standard series | 41-98 | 50-87 |
| 80°F continuous light | 69-97 | 66-98 |

The possibility suggested itself that there might be a difference in germination requirements between trees of riverine and non-riverine areas, the former possibly depending mainly on flood waters for germination and survival, and the latter on rainfall. The responses of seeds collected from the non-riverine areas of Edenhope, You Yangs, Woolhpooer, and Skipton were compared with those of provenance from riverine areas (Table 12).

An *F* test was used to compare the variances of the riverine group with the variances of the non-riverine group. The two sets of data showed no significant difference in degrees of spread. No significant difference between the mean percentage germination of riverine and non-riverine seed-lots was shown by *t* tests, hence it appears that seeds from trees in riverine and non-riverine areas do not differ in their requirements for germination.

The salient implications of the results were:

95°F was close to the optimum temperature for all seed-lots, irrespective of light conditions.

At temperatures above and below 95°F germinative energies and capacities varied widely between seed-lots.

(ii) *Lots Collected in One Locality*.—Seeds collected from each of four trees spaced at intervals of 3–4 miles along the Hamilton Road in the Skipton district were tested for variation in germination response between trees of one locality. Analysis showed that the variations between these four lots were as great as those between widely different localities. The ranges of mean percentage germination for the four Skipton seed-lots are given in Table 13; these should be compared with Table 12.

TABLE 13
RANGE OF MEAN PERCENTAGE GERMINATION OF FOUR SEED-LOTS
COLLECTED AT SKIPTON

| Treatment | Range of Mean Percentage Germination |
|-----------------------|--------------------------------------|
| 95°F standard series | 94–100 |
| 95°F continuous light | All 100 |
| 80°F standard series | 23–81 |
| 80°F continuous light | 26–86 |

Variation between seeds collected from trees a few miles apart led to the testing of seeds collected from each tree of a small compact group. Seeds from each tree in two groups of three trees in the Mildura district and two groups of four trees in the Barmah district were tested. The ranges in mean percentage germination for each group are given in Table 14.

TABLE 14
RANGE OF MEAN PERCENTAGE GERMINATION OF SEEDS FROM TREES IN SMALL COMPACT GROUPS

| Treatment | Range of Mean Percentage Germination | | | |
|-----------------------|--------------------------------------|----------|-----------|-----------|
| | Barmah 1 | Barmah 2 | Mildura 1 | Mildura 2 |
| 95°F standard series | 99–100 | 99–100 | 97–100 | All 100 |
| 80°F continuous light | 30–92 | 77–89 | 88–90 | 53–89 |

F tests comparing the variance of percentage germination of seed-lots from different localities with that of seed-lots of each compact group showed no significant difference in degrees of spread with three groups, but the variance of Mildura 1 was significantly different at the 5 per cent. level. The difference between the variance of Barmah 2 and the variance of seed-lots from different localities approached significance at the 5 per cent. level. More work is obviously necessary to clarify this point.

(iii) *Lots Collected from One Tree*.—Seeds were collected from the north, south, east, and west sides of a single tree in the You Yangs district. The germination

responses to the treatments given were strikingly uniform, implying little difference in the behaviour of seeds collected from different points on one tree (see Table 15).

F tests comparing the variance of percentage germinations of seed-lots from different localities with that of seed-lots from one tree showed a difference in degrees of spread significant at the 5 per cent. level.

TABLE 15
MEAN PERCENTAGE GERMINATION OF SEEDS COLLECTED FROM THE NORTH, SOUTH,
EAST, AND WEST SIDES OF ONE TREE

| Treatment | Mean Percentage Germination | | | |
|-----------------------|-----------------------------|-------|------|------|
| | North | South | East | West |
| 85°F continuous light | 95 | 97 | 99 | 95 |
| 70°F continuous light | 85 | 84 | 89 | 84 |

IV. CONCLUSIONS

The experiments described in this paper cannot be regarded as having completely covered the reactions to temperature and light shown by *E. camaldulensis* seeds. Many further avenues of investigation have been indicated during this research, and some aspects are worthy of further study. However, the trials have yielded valuable evidence on the germination responses of *E. camaldulensis* seeds, and they have given useful indications for the study of the germination requirements of seeds of other species.

The following conclusions have been derived from this work:

(1) There does not appear to be any relationship between environment of the site of collection and optimum conditions for germination of *E. camaldulensis* seeds. It would be desirable to ascertain whether there is any relationship between the conditions for maximum growth rate of seedlings and the climate under which the parent trees are growing. It was found that the variability in germination responses to particular treatments between seeds from trees a few miles apart in one locality is as great as in the responses of seeds from trees in widely different localities. There appears to be similar variability between seeds from trees constituting a small compact group, although the evidence here is inconclusive. Seeds from different parts of one tree give fairly uniform responses.

(2) *E. camaldulensis* seeds germinate best at a constant temperature of about 95°F. Only a short period at this temperature is required to stimulate subsequent germination at a temperature 20–30°F lower.

(3) Germination is stimulated by light. The light requirements for satisfactory germination vary considerably between seed-lots. The light requirements of all seed-lots appear to be effectively satisfied by 8 hours of light per day at a constant temperature of 95°F. These conditions can be regarded as close to optimum.

(4) The requirements for light are least at a temperature close to 95°F and increase with temperature above or below this. Temperature seems to substitute at least partially for light. Stratification reduces the requirement for light in germination.

(5) The extent of germination at temperatures above and below 95°F is modified by the light requirements of the seeds and the amount and duration of illumination, and is dependent on the inherent capacity of the seeds to germinate at infra-optimal or supra-optimal temperatures when light requirements have been satisfied.

The variation in the responses of different seed-lots under light and temperature conditions other than the optimum is very great.

V. ACKNOWLEDGMENTS

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VI. REFERENCES

- BLACK, M., and WAREING, P. F. (1955).—*Physiol. Plant.* 8: 300-16.
CLIFFORD, H. T. (1953).—*Aust. For.* 14: 17-20.
FISHER, R. A., and YATES, F. (1953).—“Statistical Tables for Biological, Agricultural and Medical Research.” 4th Ed. (Oliver and Boyd : London.)

THE ECOLOGY OF *EUCALYPTUS REGNANS* F. MUELL.: THE SPECIES AND ITS FROST RESISTANCE

By D. H. ASHTON*

[Manuscript received February 21, 1958]

Summary

The distribution, habit, and variation in *Eucalyptus regnans* F. Muell. are briefly described.

It is shown that *E. regnans* almost certainly forms hybrids with *E. obliqua*.

The seedlings of *E. regnans* are relatively frost-sensitive, and this sensitivity is increased by manuring with organic nitrogen. Under artificial frost, softened seedlings are killed at temperatures below 25–27°F, whilst well-hardened seedlings are killed at temperatures below 20–22°F. In the field the lethal frost temperature is some degrees lower.

Ecoclines exist in the *E. regnans* populations in south-central Victoria. The seedlings show a gradation in both frost resistance and growth rate along altitudinal gradients. Seedlings derived from trees growing at the edge of a frost pocket at Wallaby Creek (2000 ft) combine a relatively high frost resistance (equal to that of seedlings at 3700 ft) with a high growth rate.

I. THE SPECIES *EUCALYPTUS REGNANS*

In 1949 a study of the ecology of *E. regnans* was begun in the Wallaby Creek catchment on the Hume Range, 40 miles NNE. of Melbourne, with the aim of solving some of the problems concerned with the regeneration of a remarkably well-preserved mature forest. This study has led to an investigation of the autecology of the species.

This is the first paper of several dealing with this work.

(a) Distribution

Mountain ash, *E. regnans* F. Muell. (Plate 1), is confined to Victoria and Tasmania, where it is one of the major timber species. In Victoria it occurs in the Great Dividing Range from central to east Victoria, and in the Strzelecki and the Otway Ranges to the south-east and south-west of Melbourne respectively. Reliable fossil records of eucalypts are meagre (Maiden 1928; Beasley 1944), and hence no information on the previous range of *E. regnans* is available.

At present time this species is confined to the cool mountain regions, chiefly where deep, well-structured, loamy soils are derived from granitic, volcanic, or arkose sedimentary rocks. It is found growing chiefly in pure stands from sea-level (in Tasmania) to 3700 ft (in Victoria), with the major development on the southern- and eastern-facing slopes of ranges and plateaux from about 1000 to 2500 ft. The climate of these regions is mild, and the rainfall high (40–80 in. per annum), evenly

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distributed over the year, and appreciably supplemented by fogs and low cloud (Brookes 1950, Clifford 1953).

(b) *Habit*

E. regnans is renowned for its rapid early height growth. This is particularly marked during the first 20 years, when it may increase in height at the rate of 5–6 ft per year, and develop a spire-like crown not unlike that of a conifer. By 60–70 years the dominant trees reach most of their ultimate height and become rather flat-topped. At this age they are quite slender and, although up to 200 ft high, they may only be 3–4 ft in diameter at breast height. The tallest trees exceed 300 ft and in the past probably reached heights of about 350 ft. The tallest, authentically measured, standing tree is 322 ft, in Tasmania.* The crowns of *E. regnans* are relatively small at maturity, and the vertically hanging leaves permit as much as 30–40 per cent. of daylight to penetrate during the growing season. This high light intensity, together with good rainfall, allows the development of a rank and complex understorey, consisting of small trees, tree ferns, shrubs, ground ferns, and mesophytic herbs.

E. regnans does not coppice and is easily killed by fire, hence regeneration is dependent on seedling establishment.

(c) *History*

When these forests were discovered over 100 years ago they were tall and of even-aged appearance. They were first exploited by paling and shingle splitters, who worked through most of the forests cutting out the best and most fissile timber. In 1851 large areas of forest were devastated in most districts by the colony's first known disastrous bushfire. From about 1860–1880 most of the big forests of Gippsland were cut and burnt and the dairying industry established. The analogous Otway ranges were likewise opened up for settlement from about 1880–1900. Both of these regions suffered extensively from the serious bushfires of 1898. Since the turn of the century the *E. regnans* forests to the east and north-east of Melbourne have suffered periodically from devastating bushfires, which have reduced large areas to scrub and bracken. During the last and worst fire in 1939 almost all of the remaining mature *E. regnans* was burnt, and subsequently replaced by dense regrowth forests. Today only vestiges of the original forests remain.

(d) *Cytology*

For those eucalypt species examined the haploid number of chromosomes is 11 (Smith-White 1942; Aitchison 1947). Root tips of *E. regnans* were collected at three-hourly intervals from 9 a.m. until midnight and fixed in formalin aceto-alcohol. Squashes stained with iron aceto-carmin showed that mitosis was most active towards midnight and metaphase plates revealed that the diploid number was 22. The chromosomes at this stage were very small and appeared spherical to shortly oblong.

(e) *Variation of the Phenotype*

A close inspection of apparently uniform stands of *E. regnans* shows that there is a considerable amount of variation amongst individuals. Trees differ in form,

*Measured by R. A. Terry, licensed surveyor, Hobart, for Australian Newsprint Mills in Styx River Valley, 1956.

crown vigour, colour of shoot tips, buttress size, gum-bark pattern, size of mature fruits, and the data and duration of flowering. To what extent these differences are due to genetical or environmental factors has not yet been determined.

A marked variation in seedling growth is a most conspicuous feature of *E. regnans*, both in the field and in the more controlled environment of the glasshouse. In regeneration plots at Wallaby Creek, Vic., about 20–30 per cent. of the seedlings were relatively stunted and were soon eliminated by competition with more vigorous

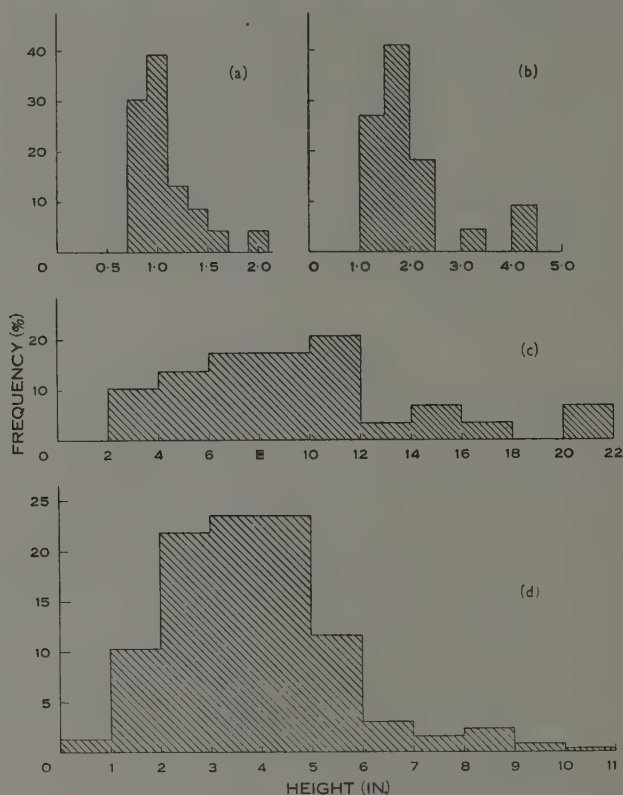


Fig. 1.—Frequency histograms of seedling heights from various experiments. (a), Variation trial, 3 months old; (b), variation trial, 4 months old; total of (a) and (b) = 23. (c), Mycorrhizal experiment, 9 months old, total = 29; (d), frost experiment, 4 months old, total = 302.

seedlings. This stunting has been called “dwarfism” by Pryor, and is found in many species and hybrids of *Eucalyptus*.

In November 1952 an experiment was set up in the glasshouse so that the variation between seedlings could be studied in a relatively uniform environment. Two hundred seeds were placed on moist cotton-wool at 75°F, and after 10–14 days 24 had germinated. About 1 week later these seedlings were transplanted, with extreme care, into 5-in. pots containing thoroughly mixed loamy soil from the *E. regnans* forests. Only one seedling was grown in each pot, and every care was taken to ensure that no lack of water occurred. The height growth, leaf number, and the

length and breadth of the largest leaf was measured after 2 and 3 months. The frequency histograms of height measurements in Figure 1 show that the distribution is skewed towards the lower height frequencies, and that relatively few seedlings are vigorous. The variation of leaf number was much less than that of height, and at 3 months ranged from six to eight. As expected, there was a fairly close correlation between leaf size and height growth.

In another experiment 29 seedlings were grown in forest soil in 1-in. diameter plywood tubes. These were packed in *Thuidium* moss and the height growth was measured after 9 months. The seedling growth was again variable. The height growth ranged from 2 to 21 in., and the frequency distribution was again skewed to the lower height classes. About 80 per cent. of the seedlings had heights less than 12 in.

In 1953 the height growth of tubed seedlings 4 months old was also measured. These seedlings were being grown for frost resistance trials and the seed came from 13 different localities in both Victoria and Tasmania. The seedlings from the different localities varied considerably both in the mean height and in the height range, and the form of the frequency distribution histogram. The pooled data for the 300 seedlings again showed a distribution skewed towards the lower height classes. The heights ranged from 0.8 to 10.2 in., with a broad peak between 2 and 5 in.

This great heterogeneity is not only a feature of seedling development, but is also found in dense, even-aged stands which arise after bushfires. It is likely that the genetical variability of *E. regnans* is a fundamental factor concerned in the rapid thinning out of dense, even-aged stands.

In young seedlings several other variable characters can be observed; the limited evidence available suggests that they are inheritable. Marked differences occur in the anthocyanin content of the hypocotyl epidermis; emerging radicles at germination are occasionally abortive or poorly developed; and in some seed batches about 1 per cent. of the seedlings develop three cotyledons. Very occasionally seedlings develop a weeping habit, and large trees with pendulous branches are known from Wallaby Creek.

Albino variegations are rare, and only three have been observed in these studies. In one seedling, the albino areas did not fully expand and deformities of the leaves occurred, whilst in another, the albinism tended to be unstable. Evidence from its distribution on the stem suggested that it was derived from a sectorial chimaera.

Variations in the frost resistance of seedlings and the flowering times of trees also occur.

(f) Hybridization

Hybridization between closely related species of *Eucalyptus* has been suspected for many years, and more recently has been confirmed by Brett (1949), Pryor (1951, 1953, 1957), and Clifford (1954).

Eucalyptus regnans F. Muell. was originally described under the name of *E. amygdalina* Labill. In 1879 Mueller recognized the tall gum-barked form or variety as *regnans*. In 1887 Mueller described this variety as a distinct species. In 1896 Deane and Maiden described the form of *E. regnans* in New South Wales and East

Victoria as *E. fastigata*, but in 1899 retracted their description in favour of merging the two species. For many years *E. fastigata* was regarded as a form or variety of *E. regnans*, but in 1928 Maiden finally decided that this form was indeed a distinct species. This decision has been accepted by Blakely (1934). Ewart (1930) regarded *E. regnans* var. *fastigata* (or *E. fastigata* Deane & Maiden) in eastern Victoria as intermediate between *E. obliqua* L'Hér. and *E. regnans* F. Muell., and suggested that it may have arisen as a result of hybridization between these two species.

E. fastigata Deane & Maiden is very similar to *E. regnans* F. Muell., but differs from it by the development of a fibrous stringy bark on the whole of the main trunk. The lateral branches remain gum-barked as in *E. regnans*. The leaves are similar to those of *E. regnans*, but tend to be slightly smaller and glossier. The fruits are small and similar to *E. regnans* except in the development of a definite domed disk and exert valves. The wood characteristics are practically indistinguishable from those of *E. obliqua* (Maiden 1928).

E. fastigata occurs widely in the southern tablelands of New South Wales, where it occupies a habitat similar to that of *E. regnans* and is, moreover, associated with many of the same understorey species. Pryor (personal communication) considers that *E. fastigata* is a uniform, well-defined species which will hybridize freely with any member of the Renantherae with which it comes in contact. Whether or not *E. fastigata* was derived from the hybridization of *E. regnans* with a stringy-bark member of the Renantherae is not known, but trees intermediate between *E. regnans* and *E. obliqua* are to be found in the ecotone between these two forests wherever they occur in Victoria and Tasmania, and at least some of these trees closely resemble *E. fastigata*.

The intermediate trees are most commonly encountered in dense young forests, and are usually rare in the mature forests where the density of trees is only 20 per acre. They are, however, more frequent in mature forest in the Florentine Valley in Tasmania, where *E. obliqua* often enters the *E. regnans* forest. In Victoria, intermediate trees tend to spread further into the *E. obliqua* forests than into the *E. regnans* forests. In two localities near the Hume Range, gum-topped stringybark intermediates have been found up to half a mile inside the *E. obliqua* boundary. The intermediate trees have, from time to time, been superficially determined as *E. fastigata*, and have been called in the Otway Ranges "gum-topped messmate" or "Otways messmate".

Intermediate trees were studied along the boundary of the *E. regnans* forest at Wallaby Creek on the Hume Range, where dense pole and spar stage stands have developed since bushfires in 1900 and 1926. On the boundary where the soil is deep *E. regnans* grows up to 20–30 ft taller than *E. obliqua*, and *E. regnans* will, if dense enough, finally suppress *E. obliqua* in part of the ecotone. The height of the intermediate trees ranges between that of the two adjacent species.

The most striking character of the intermediate trees is that of the persistence and texture of the bark. *E. regnans* possesses a subfibrous persistent bark on the butt of the tree (Plate 1). This bark occupies the lowermost 10–15 per cent. of the trunk, and extends up to 30–40 ft in mature trees. At the limiting height it becomes thinner and finer and strips off in long ribbons, exposing the smooth, greenish-grey deciduous

bark of the upper trunk and branches. *E. obliqua*, on the other hand, possesses a furrowed, fibrous, stringy bark on the trunk and the primary and secondary branches. The intermediate trees show a complete range between the characteristics of *E. regnans* and *E. obliqua*. The most conspicuous intermediates are those which resemble *E. obliqua* but possess a gum-barked crown. Trees can be found in which gum bark has developed on the secondary branches only, or all branches, or the entire crown and the upper part of the main trunk. One tree near the Cascades (Wallaby Creek) is very similar to *E. regnans* except that the bark on the butt can be torn off in strips up to 3 ft long, indicating a definite *E. obliqua* characteristic. On the divide between the Silver and Wallaby Creeks other intermediates can be found which bear subfibrous

TABLE I
CONTRASTING CHARACTERISTICS OF SEEDLINGS USED IN THE ANALYSIS OF THE
PROGENY OF INTERMEDIATE TREES

| Characteristics | <i>E. regnans</i> | <i>E. obliqua</i> |
|-----------------|---------------------|-----------------------------|
| Oil Glands | | |
| Density | Numerous | Sparse |
| Diameter | Small | Large |
| Oil Composition | | |
| Odour | Sweetish | Tang |
| Leaf | | |
| Margin | Undulate Crenate | Flat Entire |
| Surface | Smooth | Papillose |
| Lignotubers | Absent | Present in lower leaf axils |

bark like *E. regnans*, but differ from it by developing this persistent bark up the main trunk to the base of the crown, or higher. Hence, in the intermediate trees there appears to be recombination between the characters of height of persistent bark and the degree of bark fibrousness.

The fruits of the intermediates have not been studied in any detail, but most of those observed possess *E. obliqua* characteristics. *E. regnans* bears rather hemispherical fruits tapering to a pedicel, a flat rim, and a disk almost level with the rim. *E. obliqua*, on the other hand, is more elongate and constricts slightly at the top, has a sharp descending rim and sunken valves. The fruits of the intermediates are often larger than *E. obliqua* but of the same general shape. Some fruits observed have a flattened rim as in *E. regnans*. Work carried out by McLaughlin (1956) on the quantitative measurement of fruit shape in *E. obliqua* indicates that intermediate gum-topped messmates (var. *discocarpa* Blakely) from Wilson's Promontory bear affinities with *E. regnans*.

During experimental work in 1953, seedling characteristics of *E. regnans* and *E. obliqua* were studied which enabled an analysis of the intermediates to be made.

Seed was, therefore, collected from half-barked intermediate trees at Mt. Disappointment and the Otway Ranges in Victoria, and in the Florentine Valley, Tas., and seedlings were raised in the glasshouse for 6 months. Seedlings were raised simultaneously from seed collected from pure *E. regnans* stands in the centre of the Wallaby Creek Catchment, and from pure *E. obliqua* in the Daylesford area. The leaves of seedlings in their juvenile phase show continuous variation up the stem, so that comparisons were standardized by confining measurements to the leaf pair at the fifth node. Ten seedlings from each locality were selected at random for this analysis (Table 1).

The density and diameter of the oil glands (measured under the low power of the microscope) were used to obtain a strictly quantitative measure of the degree of intermediacy. The density of the oil glands was determined by counting the number

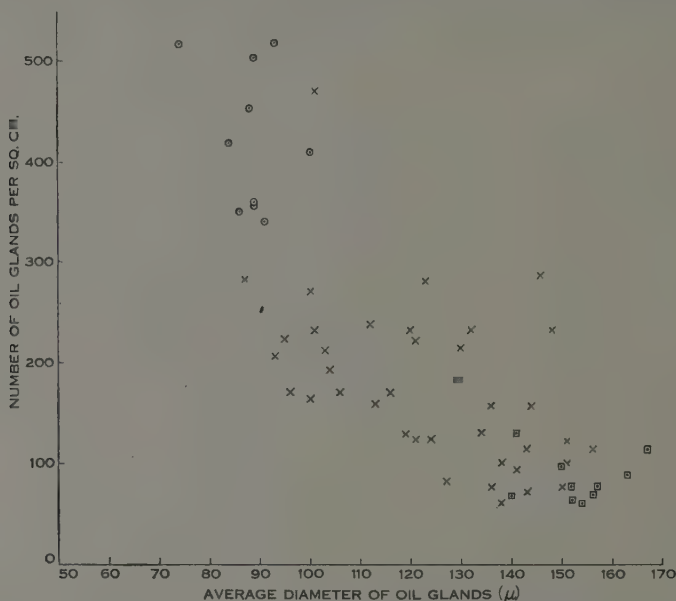


Fig. 2.—The “recombination spindle” obtained from the analysis of oil glands in the leaves of seedlings obtained from the putative parent species and the presumed hybrids. ○, *E. regnans*; ×, hybrid; □, *E. obliqua*.

present in ten fields on each leaf of the pair. The diameter was determined by measuring the oil gland nearest the centre of each field with a micrometer eyepiece. The leaf characteristics and oil odour were assessed qualitatively by assigning each seedling to one of three or four categories. The assessment of lignotuber development was delayed until the seedlings were 2 years old. The results of the analysis of the fifth-node leaves are set out in Table 2.

The oil gland density of the intermediates varies from that of *E. obliqua* to about two-thirds that of *E. regnans*. The average diameters of the oil glands of the intermediates show more variation than the densities, and range from those of *E. regnans* to those of *E. obliqua*. When the oil gland density is plotted against the average diameter of the oil glands the intermediate seedlings show a very much greater scatter than the seedlings of either *E. regnans* or *E. obliqua*. This distribution is shown in Figure 2, and resembles in outline the “recombination spindle” of Anderson (1949).

TABLE 2
ANALYSIS OF FIFTH-NODE LEAVES

| Statistic | Seedlings | Oil Glands | | | Leaf | | |
|--|-----------|---------------------------|-----------------------|-------|----------------------|-------------------|--------------------|
| | | Density per sq. cm. | Diameter (μ) | Odour | Surface Papillose | Margin Crenate | Margin Undulate |
| <i>E. regnans</i> (Wallaby Creek—Toolangi) | | | | | | | |
| | 1 | 410 | 100 | 1 | 1 | 1 | 1 |
| | 2 | 453 | 88 | 1 | 1 | 1 | 1 |
| | 3 | 360 | 89 | 1 | 1 | 1 | 1 |
| | 4 | 518 | 93 | 1 | 1 | 1 | 1 |
| | 5 | 340 | 91 | 1 | 1 | 1 | 1 |
| | 6 | 350 | 86 | 1 | 1 | 1 | 1 |
| | 7 | 504 | 89 | 1 | 1 | 1 | 1 |
| | 8 | 419 | 84 | 1 | 1 | 1 | 1 |
| | 9 | 353 | 89 | 1 | 1 | 1 | 1 |
| | 10 | 517 | 74 | 1 | 1 | 1 | 1 |
| Average | | 422.4 | 88.3 | 1 | 1 | 1 | 1 |
| Affinities (%) | | 0 | 0 | 0 | 0 | 0 | 0 |
| Coeff. of variation (%) | | 17.0 | 7.5 | — | — | — | — |

| | | | | | | | |
|--------------------------------|----|------|-------|-----|-----|-----|-----|
| <i>E. obliqua</i> (Daylesford) | | | | | | | |
| | 1 | 96 | 150 | 4 | 3 | 1 | 3 |
| | 2 | 63 | 160 | 4 | 3 | 1 | 3 |
| | 3 | 82 | 159 | 4 | 3 | 2 | 4 |
| | 4 | 68 | 156 | 4 | 3 | 1 | 4 |
| | 5 | 60 | 154 | 4 | 3 | 3 | 3 |
| | 6 | 88 | 163 | 4 | 3 | 3 | 4 |
| | 7 | 76 | 157 | 4 | 3 | 3 | 4 |
| | 8 | 68 | 140 | 4 | 3 | 2 | 3 |
| | 9 | 113 | 167 | 4 | 3 | 3 | 3 |
| | 10 | 130 | 141 | 4 | 3 | 3 | 4 |
| Average | | 84.4 | 154.7 | 4.0 | 3.0 | 2.2 | 3.5 |
| Affinities (%) | | 100 | 100 | 100 | 100 | 100 | 100 |
| Coeff. of variation (%) | | 27.1 | 5.6 | — | — | — | — |

TABLE 2 (Continued)

| Statistic | Seedlings | Oil Glands | | | Leaf | | |
|-----------|-----------|---------------------------|-----------------------|-------|----------------------|-------------------|--------------------|
| | | Density per sq. cm. | Diameter (μ) | Odour | Surface Papillose | Margin Crenate | Margin Undulate |

Hybrid (Turton's Pass, Otways)

| | | | | | | | |
|-------------------------|----|-------|-------|------|------|------|------|
| | 1 | 124 | 124 | 3 | 3 | 2 | 3 |
| | 2 | 76 | 150 | 3 | 3 | 1 | 2 |
| | 3 | 156 | 136 | 4 | 3 | 3 | 3 |
| | 4 | 164 | 100 | 3 | 1 | 1 | 1 |
| | 5 | 133 | 134 | 3 | 2 | 3 | 1 |
| | 6 | 156 | 144 | 4 | 3 | 3 | 1 |
| | 7 | 181 | 130 | 4 | 2 | 2 | 3 |
| | 8 | 232 | 132 | 3 | 3 | 1 | 3 |
| | 9 | 232 | 101 | 2 | 2 | 1 | 2 |
| | 10 | 470 | 101 | 2 | 3 | 1 | 3 |
| Average | | 192.4 | 125.2 | 3.1 | 2.5 | 1.8 | 2.2 |
| Affinities (%) | | 68.1 | 55.6 | 70.0 | 75.0 | 66.0 | 48.0 |
| Coeff. of variation (%) | | 56.9 | 14.7 | — | — | — | — |

Hybrid (Carisbrook Creek, Otways)

| | | | | | | | |
|-------------------------|----|-------|-------|------|------|------|------|
| | 1 | 127 | 119 | 3 | 3 | 1 | 1 |
| | 2 | 76 | 136 | 1 | 1 | 1 | 1 |
| | 3 | 60 | 138 | 1 | 1 | 1 | 1 |
| | 4 | 283 | 87 | 1 | 1 | 1 | 1 |
| | 5 | 82 | 127 | 4 | 3 | 2 | 3 |
| | 6 | 93 | 141 | 4 | 3 | 2 | 3 |
| | 7 | 159 | 113 | 4 | 3 | 2 | 2 |
| | 8 | 124 | 121 | 4 | 2 | 2 | 2 |
| | 9 | 238 | 112 | 4 | 1 | 3 | 2 |
| | 10 | 232 | 120 | 2 | 2 | 3 | 3 |
| Average | | 147.4 | 121.4 | 2.8 | 2.0 | 1.8 | 1.9 |
| Affinities (%) | | 81.4 | 49.9 | 60.0 | 50.0 | 66.0 | 36.0 |
| Coeff. of variation (%) | | 53.0 | 13.0 | — | — | — | — |

TABLE 2 (Continued)

| Statistic | Seedlings | Oil Glands | | | Leaf | | |
|---|-----------|---------------------------|-----------------|-------------------|----------------------|-------------------|--------------------|
| | | Density per sq. cm. | Diameter (μ) | Odour | Surface Papillose | Margin Crenate | Margin Undulate |
| Hybrid (Mt. Disappointment, Hume Range) | | | | | | | |
| | 1 | 280 | 123 | 1 | 1 | 3 | 1 |
| | 2 | 223 | 95 | 1 | 1 | 1 | 1 |
| | 3 | 181 | 129 | 2 | 2 | 1 | 2 |
| | 4 | 272 | 100 | 3 | 2 | 3 | 3 |
| | 5 | 212 | 103 | 1 | 1 | 1 | 1 |
| | 6 | 170 | 96 | 3 | 1 | 3 | 3 |
| | 7 | 170 | 116 | 2 | 1 | 2 | 2 |
| | 8 | 206 | 93 | 2 | 1 | 3 | 2 |
| | 9 | 184 | 117 | 3 | 2 | 2 | 3 |
| | 10 | 221 | 121 | 2 | 2 | 3 | 2 |
| Average | | 219.9 | 109.3 | 2.0 | 1.4 | 2.2 | 2.4 |
| Affinities (%) | | 59.9 | 31.7 | 33.3 | 32.2 | 100.0 | 56.0 |
| Coeff. of variation (%) | | 18.2 | 12.2 | — | — | — | — |
| Hybrid (Florentine Valley, Tas.) | | | | | | | |
| | 1 | 71 | 143 | 1 | 1 | 1 | 2 |
| | 2 | 192 | 104 | 1 | 2 | 1 | 2 |
| | 3 | 113 | 156 | 1 | 1 | 1 | 2 |
| | 4 | 99 | 151 | 1 | 1 | 1 | 2 |
| | 5 | 286 | 146 | 4 | 3 | 1 | 3 |
| | 6 | 100 | 138 | 4 | 2 | 2 | 3 |
| | 7 | 232 | 148 | 3 | 3 | 1 | 2 |
| | 8 | 122 | 151 | 3 | 3 | 1 | 2 |
| | 9 | 113 | 143 | 4 | 3 | 3 | 2 |
| | 10 | 215 | 130 | 4 | 1 | 3 | 4 |
| Average | | 154.3 | 141.0 | 2.6 | 2.0 | 1.5 | 2.4 |
| Affinities (%) | | 79.3 | 79.4 | 53.3 | 50.0 | 41.7 | 56.0 |
| Coeff. of variation (%) | | 46.3 | 10.6 | — | — | — | — |
| | | All hybrids | | <i>E. regnans</i> | | <i>E. obliqua</i> | |
| Coeff. of variation of density (%) | | 43.6 | | 17.0 | | 27.1 | |
| Coeff. of variation of diameter (%) | | 12.6 | | 7.5 | | 5.6 | |

The results also show that the intermediates examined bear a closer affinity to *E. obliqua* than to *E. regnans*. This may be due to the fact that the trees chosen as seed source were those of the gum-topped messmate type. Most intermediate seedlings possessed oil glands more numerous and somewhat smaller than *E. obliqua*, and an oil odour which was somewhat milder than that of *E. obliqua*. However, sometimes the reverse condition was found, in which there was a high density of large oil glands or a low density of small oil glands. Also, the variation of oil gland size in the leaves of the intermediates was greater than in either of the two species considered. When the oil gland measurements were examined in conjunction with the qualitative characters of leaf margin and leaf surface and oil composition, it was apparent that there were definite recombinations giving a wide variation in the phenotype of the intermediates. Thus, intermediate seedlings with *E. obliqua*-type oil glands may possess the oil odour of *E. regnans* and vice versa. The morphological leaf characteristics were also recombined with the oil gland and oil odour characteristics. Photographs of oil glands and leaf surface features of parent species and suspected hybrids are shown in Plate 2.

TABLE 3
COMPARISON BETWEEN LIGNOTUBER DEVELOPMENT AND OIL GLAND CHARACTERS

| Comparison | <i>E. regnans</i> | <i>E. obliqua</i> | Intermediates | |
|-------------------------------|-------------------|-------------------|---------------|-------------------|
| | | | Otways | Florentine Valley |
| Quantitative | | | | |
| Average number of lignotubers | 0 | 4.2 | 3.2 | 3.0 |
| Qualitative | | | | |
| Oil odour score | 1.0 | 4.0 | 3.1 | 2.5 |
| Oil gland density score | 1.0 | 3.0 | 2.5 | 2.2 |

The results of the assessment of the lignotuber development again indicated that the intermediate seedlings were more variable than either of the two species considered (Table 3). Lignotuber development occurred in all of the *E. obliqua* seedlings, most of intermediate seedlings, and in none of the *E. regnans* seedlings. The lignotubers are best developed in the axils of the cotyledons, where, in seedlings of this age, they reach diameters of up to $\frac{1}{4}$ in. In those seedlings where the development is most pronounced the swellings may occur in the axils of each succeeding pair of juvenile leaves up to the fourth node. In general the lignotuber development diminishes with each succeeding node up to the fourth node, where it virtually ceases. In those seedlings in which the development of lignotubers is poor, swellings may only occur at the cotyledonary node, and may only occur in one axil. In *E. obliqua* seedlings the lignotuber development was moderate to heavy, and the number varied from two to eight per plant and averaged about four. In the intermediate seedlings the lignotuber development was variable in size, and ranged in number from nil to eight per plant. The number of lignotubers developed was found to be independent of the total height and degree of suppression of the crown of the seedling. About 9–10 per cent. of the inter-

mediate seedlings were totally devoid of lignotuber swellings, and in this respect resembled *E. regnans*. However, most of these seedlings possessed an oil odour resembling that of *E. obliqua*.

The evidence accumulated from this study indicates that the intermediate trees have almost certainly arisen by hybridization between *E. regnans* and *E. obliqua*. The range of affinities towards either parent could be due to repeated backcrossing. The flowering times of the putative parents only just overlap in early autumn, and it is possible that the extent and frequency of hybridization could be affected by seasonal conditions. It is likely that the chances of hybridization between these species would be greater if heavy flowering in both species were to coincide. This did occur in 1956; however, the results of any hybridization would not normally become evident unless considerable regeneration, such as follows heavy bushfires, took place. *E. obliqua* flowers from January to mid March with a maximum in February, whilst *E. regnans* flowers from mid March to early July with a maximum in April and May. The present studies suggest that these eucalypt flowers are protandrous, hence there would be a tendency for the early ripening pollen of *E. regnans* flowers to be transferred to the late ripening stigmas of *E. obliqua*. This may account for the tendency of *E. regnans* characters to infiltrate into *E. obliqua* forests for considerable distances, although definite conclusions on this point would require further study.

The oblique leaf base is often regarded as a characteristic of *obliqua*. However, *E. regnans* may also produce this type of leaf, particularly in the post-juvenile stages of the seedling. A preliminary assessment of the frequency of the oblique leaf character in the adult foliage of the tree crowns was made by collecting branches at different distances from an *E. obliqua* boundary following a severe storm in May 1954. The percentage of leaves with the oblique base was determined and the distance of the site of collection noted. Although the results were variable, they suggested that there was a tendency for the prevalence of the oblique leaf to increase as the *E. obliqua* boundary was approached. The percentage of oblique-based leaves increased from 15–20 per cent. 1 mile from the *E. obliqua* boundary to 25–30 per cent. at the boundary. Although work on this aspect is incomplete, it suggests that either there has been an introgression of this characteristic from the *E. obliqua* forests into the *E. regnans* forests, or that the prevalence of the oblique leaf is the result of a habitat gradation. If it is due to introgression it is possible that less obvious physiological characteristics may likewise have been introduced. This could have a bearing on many problems in both silviculture and ecology.

Pryor (1957) considers that the Renantherae belong to a definite breeding group in the eucalypts which do not hybridize with other groups. Other species of the Renantherae which occur in the neighbourhood of *E. regnans* forests in Victoria are *E. gigantea* Hook.f., *E. radiata* Sieb., *E. dives* Schauer, *E. sieberiana* F. Muell., and *E. macrorrhyncha* F. Muell. Detailed work has not been extended to the upper altitudinal margin of the *E. regnans* forests where *E. gigantea* occurs, but since its flowering period is similar to that of *E. obliqua* it is also likely to hybridize occasionally with *E. regnans*. It is unlikely that either *E. radiata*, *E. dives*, or *E. sieberiana* would hybridize with *E. regnans* since the flowering period of these species ranges from October to January and is, therefore, widely different from that of *E. regnans*. In addi-

tion, these species rarely occur closer to the *E. regnans* boundary than a quarter of a mile, and are usually found on relatively dry sites in the *E. obliqua* forests. Specimens* collected by H. T. Clifford and C. G. Elliott from the Cathedral Range at Buxton are intermediate between *E. regnans* and *E. macrorrhyncha*. These two species normally occupy very contrasted and widely separated habitats. However, they do grow close together at Sugarloaf Saddle, and since their flowering times overlap slightly, it is likely that they do occasionally hybridize. *E. goniocalyx* F. Muell. and *E. nitens* Maiden flower at about the same time as *E. regnans* and mix to some extent with it—the former species below 2500 ft and the latter above 2500 ft. However, these species are members of the *Parallelantherae* and are, therefore, unlikely to cross with *E. regnans*.

II. THE FROST RESISTANCE OF EUCALYPTUS REGNANS

In view of the importance of frost in the plateau areas in central and east-central Victoria, a preliminary study was carried out on the effect of frost on seedlings of *E. regnans*. Seedlings were chosen for study because they are, under field conditions, subjected to the severest frosts on any site.

(a) Field Observations

R. Oldham and J. Redmond, Forest Officers of the Melbourne and Metropolitan Board of Works, have carried out extensive reafforestation operations in bracken and scrubland in the Wallaby Creek area, and noted that young seedlings were killed or crippled wherever they were transplanted into flat or low-lying grassy areas. Field experiments carried out by Redmond showed that *E. regnans* and young bracken are similarly affected by spring frosts and that the presence of grass tussocks can be taken as an indicator of frosty sites. The grassland and bracken clearings in the *E. regnans* area of the Hume Plateau have been produced by repeated early fires together with the activities of paling splitters (Plate 3).

Field observations carried out in 1950–51 confirmed the views of these workers, and suggested that frost was also a major factor preventing the natural invasion of *E. regnans* into these grassy plains and depressions. In late autumn and winter cold air often forms a low mist in such areas.

Regeneration of *E. regnans* occurs slowly around the edges of these grasslands, particularly where the more frost-resistant silver wattle (*Acacia dealbata* Link.) has established and acted as a “nurse crop” (Plate 3). A *Poa caespitosa* J. Forst. tussock grassland at 2000 ft was selected for study, and maximum and minimum thermometers were placed 2 in. above the ground surface between the tussocks at the centre and the edges of the frost hollow. In midwinter the minimum temperatures in the middle of the grassland frequently fell to 10–12°F and occasionally to 4–5°F. Ground frosts of 27–28°F often occurred during summer whenever the conditions were suitable. The corresponding temperatures at the bracken–grassland margin were very much higher and seedlings of *E. regnans* growing in these sites were subjected to winter minimum temperatures of 20–23°F. Thermograph records made in these areas indicated that the

*Now in the Melbourne University Herbarium.

temperature fell below freezing point soon after sunset and fell steeply for 5–6 hours and then more gradually until sunrise. On still, cloudless nights the severe frost temperatures usually lasted for 6–8 hours, and the total period below 32°F was not less than 12 hours. In early July 1951, seedlings growing near parent trees at the bracken margin of the grassland were carefully transplanted to the centre of the frost hollow. In the succeeding week of mild and sometimes wet weather no damage to the seedlings was noticed. However, when frosty conditions again set in these seedlings were killed outright. Thermometer records indicated that the minimum temperatures sustained at 2 in. above the ground were 14–16°F. Although there were no transplant controls made at the margin of the pocket, the seedlings remaining there were not damaged, and it was concluded that frost was a most important factor in the failure of regeneration of these areas.

(b) *Preliminary Refrigerator Experiments*

(i) *Method*

Tubed seedlings obtained from the Wallaby Creek nursery in July 1953 were used for these experiments. One-half of the stock had been previously manured with blood and bone according to the standard nursery practice. The seedlings were placed in an unheated glasshouse at Melbourne in order to prevent damage from possible severe frosts in July. Some growth had taken place when the experiments were carried out, and the manured seedlings had grown at a faster rate than the controls. The seedlings were in a rather softened condition, as the minimum temperatures in the glasshouse rarely fell to 33–34°F and the maximum temperatures frequently reached 70–80°F. The seedlings were 6–8 in. high, and ranged in development from the fourth to the tenth leaf stage. Three seedlings from both the manured and control batches were selected at random for each trial, and packed in sawdust in a glazed porcelain pot to prevent freezing of the roots.

(ii) *Assessment of Frost Damage*

A scheme of scoring was evolved in which the damage to both the leaves and the axillary buds was taken into account. It was felt that the power to recuperate by the development of axillary buds was as important as the damage sustained by the leaves, hence the score was divided equally between bud and leaf damage.

(1) *Leaf damage*.—This was assessed by taking each leaf separately and estimating the fraction of leaf area killed to the nearest quarter. The average leaf damage for each plant could then be calculated, e.g.;

| | | | | | |
|------------------------------------|---|---------------|---------------|---------------|---|
| Fractions of leaf killed | 1 | $\frac{3}{4}$ | $\frac{1}{2}$ | $\frac{1}{4}$ | 0 |
| Numbers of leaves in each category | 4 | 2 | 1 | 1 | 0 |
| Total number of leaves | 8 | | | | |
| Total damage | $(4 \times 1 + 2 \times \frac{3}{4} + 1 \times \frac{1}{2} + 1 \times \frac{1}{4}) = 6.2$ | | | | |
| Average damage for plant | $6.2/8 = 0.77$ | | | | |

(2) *Bud damage*.—The bud damage was assessed directly as the proportion of total buds killed. The number of buds killed could usually be determined soon after

frosting, but doubtful cases were checked 1–2 weeks later when axillary buds had commenced to grow. An example follows:

| | |
|-----------------------|---------------|
| Number of buds killed | 5 |
| Total number of buds | 17 |
| Proportion killed | $5/17 = 0.29$ |

(3) *Total damage*.—As the two assessments above were given equal weight the total damage was expressed as the average of the two and converted to a percentage, e.g.:

| | |
|-------------|---|
| Leaf damage | 0.77 |
| Bud damage | 0.29 |
| Average | $[(0.77 + 0.29)/2] \times 100 = 53$ per cent. |

(iii) *Experiments*

Seedlings were brought into the laboratory from the glasshouse and, after 1 hour, were placed in the lower compartment of the refrigerator, in which freezing temperatures could be maintained with a temperature fluctuation of 2° F. In this position only a gentle air current circulated over the seedlings. After frosting the seedlings were placed in the laboratory for a further 2 hours before being replaced in the glasshouse.

(1) *The killing temperature*.—This temperature is not fixed, but varies greatly with the conditions of the experiment. In an initial test, plants were placed in the refrigerator for 4 hours in order to gauge the range of the killing temperatures under these conditions. The damage at 28–30°F was slight, at 27–29°F it was moderate, and at 25–27°F it was very heavy. No seedlings survived below 25°F. It is probable that both the softened condition of the seedlings and the regular rhythmical fluctuation of the refrigerator temperature contributed greatly to the relative mildness of the killing temperature.

(2) *The period of freezing*.—Batches of seedlings were placed in the refrigerator for periods of 2, 4, and 8 hours. In order to standardize procedure, seedlings were frozen and thawed rapidly by transferring them directly to and from the cold room.

The results are set out in Figure 3, and show a linear relationship between the frost damage and the duration of the frost. Both the percentage damage and the rate of increase of damage were greater at 25–27°F than at 27–29°F.

(3) *The rate of freezing and thawing*.—Slow freezing was achieved by placing the seedlings in the refrigerator at 34–35°F and gradually lowering the temperature to the desired frost over a period of 4 hours. Slow thawing was carried out by raising the temperature to 34–35°F over the same period of time. Most of the experiments were begun during the morning; this may have rendered the plants more sensitive owing to the lower sugar content of the leaves (Levitt 1956).

The results of these experiments were conflicting. In July a frost of 27–29°F maintained for 8 hours gave 40 per cent. damage when both freezing and thawing were slow; when either freezing or thawing, or both, were fast the damage was much greater (62–67 per cent.). On the other hand, in August, under the same conditions, most of the seedlings were killed. In later work it was decided to standardize the procedure and to use a fast freezing and thawing regime.

(4) *The age of the seedling.*—A preliminary test was carried out in August to investigate the frost resistance of seedlings of various ages ranging from 3 weeks to 18 months. The youngest seedlings were 1 in. high at the cotyledonary stage, and the oldest were 30 in. high with semi-adult foliage. The resistance of the seedlings appeared to be lowest at the 6–18 leaf stage, and was surprisingly high at the two-leaf and cotyledonary stages. The lowest leaves of the tall seedlings were often resistant, and those 8 in. above the soil were usually killed. It is possible that heat flow from the pots could result in slightly higher temperatures near the surface of the soil. If this were so, it would be in direct contrast to radiation frosts in the field, where the minimum temperatures are recorded just above the radiating surface.

(5) *The effect of hardening.*—Seedlings were placed in the open during July for periods of 14 and 30 days, during which time the minimum temperature frequently fell to 29–32°F. The seedlings were then frosted in the refrigerator at 25–27°F for 8 hours. The importance of hardening is clearly shown in Table 4, and in the experiments in 1954 hardening was allowed to continue for at least 2 months.

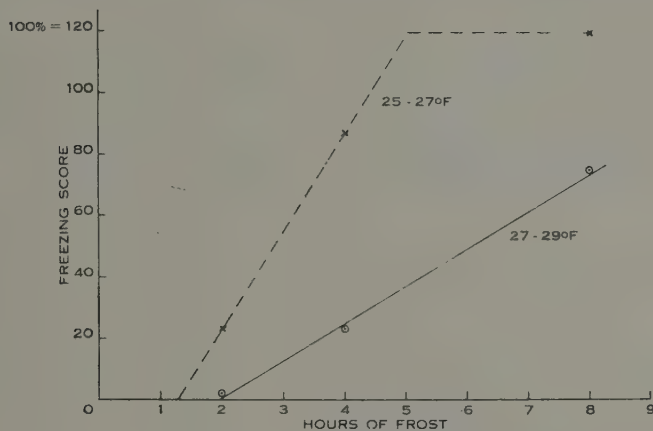


Fig. 3.—The relationship between frost damage and period and temperature of frost.

(6) *The effect of blood and bone manure.*—At the completion of the experiments the damage sustained by the control and manured seedlings was analysed. In most cases the manured seedlings were more severely damaged than the controls (Table 5). The results vary, however, and greater replications would be necessary to establish these conclusions.

The cause of the lessened resistance is not known, but the results suggest that heavy manuring of seedlings in plantation practice may have to be discontinued where conditions are frosty.

(c) *The Effect of Provenance on Frost Resistance*

In the course of the study of frost damage in 1953 it was realized that artificial frosting experiments would be of most value if relative rather than absolute frost resistance was measured. Absolute measurements of frost resistance are liable to be

affected by the degree of hardening of the seedling, the rates of freeze and thaw, and the temperature fluctuation in the refrigerator.

Day (1951) studied the frost resistance of *Larix europea* seedlings from seed sources at various altitudes and latitudes in Europe and Japan by means of artificial

TABLE 4
PERCENTAGE DAMAGE TO HARDENED SEEDLINGS AT
25-27°F

| Frosting Time (hr) | Hardened | | Unhardened |
|--------------------------|----------|---------|------------|
| | 14 days | 30 days | |
| 4 | 0 | 0 | 75% |
| 8 | 100% | 72% | 100% |

frost in a refrigerator. The results of this work indicated the existence of physiological races which possessed different degrees of frost hardiness and delayed periods of bud burst. Increased resistance occurred with increase of either altitude or latitude of seed source. Those races possessing delayed bud burst were found to be invaluable in localities where there was a likelihood of exceptional or late frosts.

TABLE 5
PERCENTAGE DAMAGE SUSTAINED BY MANURED AND CONTROL SEEDLINGS

| Temperature (°F) | Period (hr) | Freeze | Thaw | Time | Damage (%) | |
|---------------------|----------------|--------|-----------|-------|------------|---------|
| | | | | | Control | Manured |
| 27 | 8 | slow | slow | day | 40 | 40 |
| 27 | 8 | slow | fast | day | 33 | 100 |
| 27 | 8 | fast | slow | day | 41 | 87 |
| 28 | 8 | fast | fast | day | 0 | 43 |
| 27 | 4 | fast | fast | day | 0 | 73 |
| 27 | 2 | fast | fast | day | 0 | 2 |
| 25 | 2 | fast | fast | day | 0 | 40 |
| 27 | 4 | fast | very fast | day | 18 | 12 |
| 27 | 4 | slow | very fast | day | 96 | 95 |
| 27 | 8 | fast | fast | night | 23 | 97 |
| 25 | 8 | fast | fast | day | 41 | 100 |
| 25 | 8 | slow | slow | day | 39 | 53 |

(i) Method

Seed was obtained from several districts in Victoria and Tasmania. From 20 to 30 seedlings from each seed source were pricked off into the native forest soil in 2-in. diameter plywood tubes, and placed in the glasshouse. These seedlings were grown

from the two-leaf stage in September to the six-leaf stage in December, when they were packed in boxes lined with *Thuidium* moss and placed outside in the open to harden until the winter. With the onset of the cooler weather frequent small applications of blood and bone manure were necessary to prevent excessive reddening of the leaves and the development of necrotic patches. Preliminary experiments were carried out using the same refrigerator as in 1953; however, the temperature variation could not be reduced below 3–4°F and all seedlings tested died when the minimum temperature reached 26°F. This sensitivity of the seedlings was probably due to the repeated freezing and thawing of the tissues caused by the wide temperature fluctuation of the refrigerator.

The main experiment was, therefore, carried out in a cold room, where a temperature fluctuation of 1°F was maintained.

The ten best seedlings from each district were packed in boxes 2 ft long and 1 ft deep and wide, and their roots insulated from the frost by 1–2 in. of sawdust. This insulation was successful, since only the top half-inch of soil was frozen in the most severe frosts. It was also found necessary to protect the seedlings from the wind generated by the fans in the cold room, for although the velocity on the floor was only 1.3 m.p.h., and the relative humidity 82 per cent., increased desiccation of the plants occurred. The damaging effect of a cold, dry wind was discovered when the plants, which had been thoroughly watered, were placed in a refrigerator for 48 hours to harden off. The wind velocity generated by the fan was 2.5 m.p.h., and the relative humidity was only 32–35 per cent., but very severe damage was sustained by those plants in the path of the wind.

The main frost experiment was carried out in early August, when the plants had been hardened by several weeks of cool weather during which night temperatures were frequently as low as 31–33°F and occasionally as low as 28°F. The seedlings were brought in from outside at 10 p.m., and placed in the cold room at 26°F. They were removed at 10 a.m. the next morning and taken outside to their position in the open. This frost resulted in only slight damage, which was confined to the young tips. Two successive frosts at this temperature resulted in only a slight increase in damage. It was, therefore, decided to subject the seedlings to consecutive frosts of increasing intensity until about 50 per cent. damage occurred. In this way a temperature could be found which would differentiate between the seedlings of high and low resistance to a maximum degree. The 24°F frost caused light to moderate damage to the mature leaves, but was considered to be not heavy enough. The damaged and dead leaves could be distinguished a short time after having been brought out of the cold room by their darker colour and flaccid appearance. As in the 1953 experiments, the variations between seedlings were marked. Some seedlings were heavily damaged, whilst others in the same batch were not damaged at all. The 22°F frost resulted in moderate to heavy damage in most seedlings and, as 20°F was a completely lethal temperature under these conditions, it was decided to terminate this series of experiments at 22°F frost.

Meteorological conditions were measured in the vicinity of the seedlings both before and after each frosting, and are summarized in Table 6.

(ii) *Results*

The damage was assessed on the day following each frost; however, the heavy damage of the final frosting necessitated a delay of some weeks to allow some of the bud damage estimations to be made. The percentage damage for each locality is summarized in Table 7.

The correlation between frost damage at 24°F and 22°F and the altitude of seed source is illustrated in Figure 4. The gradient of the regression lines calculated for the data of each frost increased with the severity of the frost. The gradient for the 22°F frost was almost twice that of the 24°F frost, and no correlation existed in the light 26°F frosts. According to the trend line, a rise of 1000 ft in the altitude of the seed source would result in a decrease of 12 per cent. in the damage at 22°F, and of 6 per cent. of the damage at 24°F. Therefore, it is possible that the greater the frost the greater will be the differentiation between the seed sources from different altitudes. More work, however, is required before definite predictions can be made.

TABLE 6
THE ENVIRONMENT OF SEEDLINGS IN THE FROST EXPERIMENTS

| Date (1954) | Frost (°F) | Period (hr) | Temp. Prior to Frost (°F) | Room Temp. after Frost (°F) | Outside Temp. after Frost (°F) | Relative Humidity (%) | Wind Velocity | Light |
|----------------|---------------|----------------|---------------------------------------|---|--|-----------------------------|------------------|-----------------|
| 25.viii | 26 | 12 | 38 | 45 | 52-59 | 50 | Calm | Sunny |
| 26.viii | 26 | 12 | 45 | 50 | 57-60 | 48 | N., 3 m.p.h. | Sunny |
| 27.viii | 26 | 12 | 45 | 55 | 60 | 47-49 | N., 4 m.p.h. | Hazy sun |
| 28.viii | 24 | 12 | 58 | 58 | 62 | 63 | Calm | Broken cloud |
| 29.viii | 22 | 12 | 53 | 60 | 60-62 | 64 | S., 2½ m.p.h. | Broken cloud |

The residual variance about the regression lines was calculated for each frost and equalled 4.17 per cent. for 22°F and 1.83 per cent. for 24°F. The variation amongst the individuals of each locality was relatively high, and both sensitive and resistant seedlings were noted. The variation from the regression lines is to be expected, since in most cases the seed was collected from only one or two trees. However, since the flowers are normally cross-pollinated, the seed samples may still be held fairly representative of their particular locality.

The Tasmanian seed* showed surprisingly low frost resistance, particularly one north-east sample, which showed considerable damage even at 26°F. It is unfortunate that details of these localities were unobtainable, but it is possible that seed was collected from quite low altitudes. *E. regnans* grows in some areas only 50 ft above sea-level on the eastern coast of Tasmania, and, in addition, the north-east area is

* By courtesy of the Tasmanian Forestry Commission.

affected to some extent by the warm eastern Australian sea current, which may render these coastal areas less liable to severe frosts than other districts.

An interesting feature of the experiments is the relatively high resistance of seedlings raised from seed trees bordering a *Poa caespitosa* frost pocket at Wallaby Creek. The altitude of this locality is only 2050 ft, yet the percentage damage sustained by the seedlings is equal to, or only slightly greater than, that of seedlings from Lake Mountain at 3650 ft. The percentage damage is summarized in Table 8.

TABLE 7
PERCENTAGE DAMAGE SUSTAINED BY SEEDLINGS FROM DIFFERENT SEED SOURCES

| Locality | Altitude (ft) | Damage after Successive 12-hour Frosts (%) | | | | | Height Growth (in.) | |
|--------------------|------------------|---|------|------|-------|-------|------------------------|--------------|
| | | 26°F | 26°F | 26°F | 24°F | 22°F | Dec. 1953 | Aug. 1954 |
| Belgrave | 800 | 10.5 | 10.5 | 12.5 | 19.0 | 60.0 | 2.74 | 13.9 |
| Kallista | 1100 | 2.5 | 3.0 | 3.5 | 18.0 | 52.0 | 3.71 | 15.6 |
| Yarram | 1250 | 2.5 | 2.5 | 3.0 | 10.5 | 47.5 | 3.56 | 14.4 |
| Carisbrook Creek | 1300 | 5.5 | 5.5 | 5.5 | 13.5 | 56.5 | 4.92 | 18.6 |
| Turton's Pass | 1900 | 4.5 | 4.5 | 7.0 | 12.0 | 52.5 | 5.42 | 16.2 |
| Wallaby Creek | | | | | | | | |
| Frost Hollow | 2050 | 0.5 | 0.5 | 0.5 | 2.0 | 30.5 | 3.8 | 14.9 |
| Mt. Disappointment | 2350 | 2.0 | 2.0 | 2.0 | 6.5 | 44.0 | 3.10 | 15.2 |
| Silver Creek | 2550 | 2.5 | 2.5 | 3.0 | 5.0 | 35.5 | 3.84 | 16.9 |
| Ada River | 2650 | 3.0 | 3.0 | 4.5 | 7.5 | 36.0 | 4.12 | 14.3 |
| Lake Mountain, | | | | | | | | |
| Tasmania | 3650 | 0.5 | 0.5 | 2.5 | 2.5 | 28.0 | 2.71 | 11.0 |
| N.E. Tasmania | ? | 1.0 | 3.5 | 3.5 | 12.0 | 73.5 | 3.68 | 17.7 |
| N.E. Tasmania | ? | 25.0 | 26.0 | 27.5 | 27.5 | 62.0 | 5.33 | 13.9 |
| E. coast, Tasmania | ? | 0 | 2.0 | 2.0 | 6.5 | 62.0 | 1.87 | 16.1 |
| Total | | 60.0 | 66.0 | 77.0 | 142.5 | 640.0 | 48.80 | 198.7 |
| Average | | 4.6 | 5.1 | 5.9 | 11.0 | 49.3 | 3.75 | 15.3 |

The damage is, therefore, about 14 per cent. lower than expected from a normal site on "air-drained" slopes.

In many seed sources there was a high coefficient of variation (30–80 per cent.) amongst seedlings, some being killed or severely crippled, whilst others remained undamaged. This inherent heterogeneity of the population would permit the selection of the more resistant strains if severe frost conditions were imposed. It is, therefore, probable that the greater resistance of the frost hollow seedlings is due to selection from the available population when the frost hollow grasslands were formed 50–70 years ago by logging and repeated bushfires. The plants regenerating after repeated fires in this area from 1898 to 1926 have probably been subjected to rigorous selection by frost in the flats and depressions on the plateau.

(d) *Further Evidence of an Ecocline in Eucalypts
along an Altitudinal Gradient*

Since a fairly definite relationship exists between frost resistance and the altitude of seed source, it is likely that other physiological responses might also vary. The height growth of seedlings was, therefore, measured in December 1953 and again in August 1954 prior to frosting. The average heights have been included with frost damage data in Table 7. The coefficient of variation within each locality is fairly low,

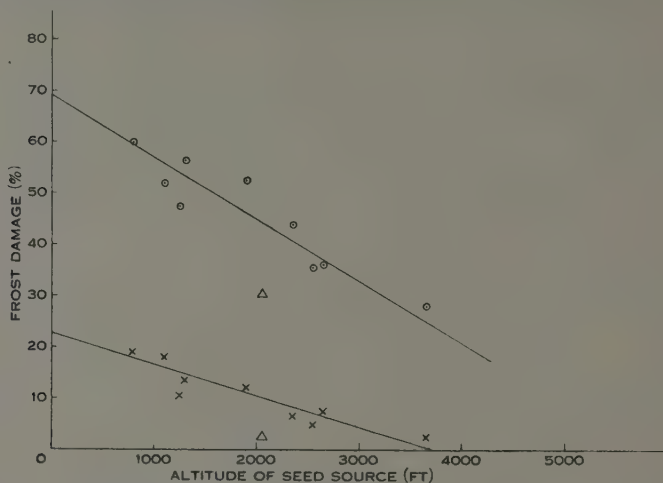


Fig. 4.—The relationship between the frost damage and the altitude of seed source. ○, 22°F frost; ×, 24°F frost; △, frost hollow seedlings.

but a considerable amount of scatter occurs between localities when the heights are plotted against altitude of seed source. However, a trend is suggested in which the greatest growth occurs in seedlings whose seed source lies in an altitudinal range of

TABLE 8
PERCENTAGE DAMAGE OF SEEDLINGS FROM A FROST HOLLOW,
COMPARED WITH OTHER LOCALITIES IN VICTORIA

| Locality | Altitude (ft) | Damage (%) | |
|---------------|------------------|------------|----------|
| | | 22°F | 24°F |
| Belgrave | 700 | 60 | 19 |
| Lake Mountain | 3650 | 28 | 4 |
| Frost Pocket | 2050 | 30 | 4 |
| Expected | 2050 | 44 ± 3 | 10 ± 1.8 |

1200–2500 ft. A curve tentatively drawn through these points shows an asymmetry towards the lower elevations, the smallest seedlings occurring from the Lake Mountain seed at 3700 ft (Fig. 5).

A similar trend of growth rate has been thoroughly studied by Duffield, Mirov, and Liddicoet (1952) in the Sierra Nevadas in California. The growth rate of *Pinus*

ponderosa seedlings from seven altitudinal seed sources (125 ft to 6919 ft) was measured in nurseries at four different altitudes (960 ft to 5650 ft) over a 12-year period. The results of this work indicate that seed from 1500–3500 ft produced seedlings with superior growth rate at each nursery. The overall growth of seedlings from all seed sources decreased with increase in altitude of the nursery. At the end of the 12-year period at the 5650 ft plantation the degree of differentiation between the various seed sources had diminished.

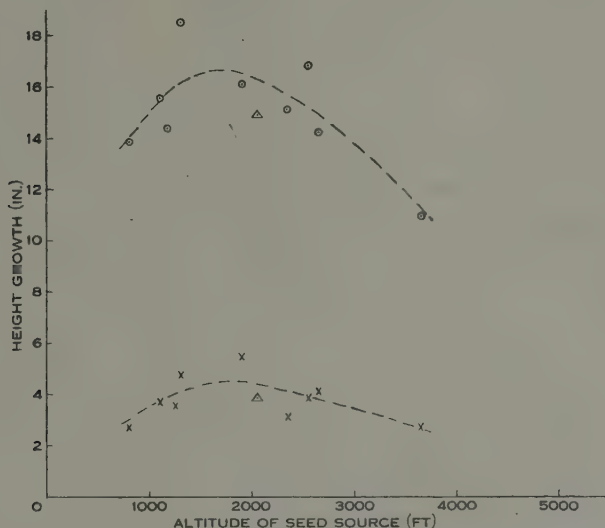


Fig. 5.—The relationship between the average height of seedlings and the altitude of seed source. ×, 4 months old; ○, 12 months old, Δ, frost hollow seedlings.

Widely distributed populations of *Achillea millifolia*, *Potentilla glandulosa*, and *Viola tricolor* have been studied by Clausen, Keck, and Heisey (1948) in California, and distinct altitudinal races or ecotypes have been described which indicate the wide range of morphological and physiological variation which exists in these species. Each ecotype was found to be physiologically adapted to its environment, owing to the development of resistance to cold and the synchronization of growth and flowering behaviour with the seasonal climatic pattern.

Gregor (1944) has, however, pointed out that the physiological and morphological variation in species is often continuous, and where this variation occurs along an environmental gradient it may be termed an ecocline. It appears, therefore, that ecoclines exist in the *E. regnans* populations in the mountains of south-central Victoria. This is illustrated by the gradation in both frost resistance and growth rate along altitudinal gradients. It is probable that further research on growth form and behaviour of seedlings from different seed sources would lead to a better understanding of many problems of distribution and competition.

Seed from the frost hollows at Wallaby Creek (2000 ft) may be of considerable importance in reafforestation in frosty localities, since the seedlings combine a relatively high frost resistance with a relatively fast height growth rate.

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IV. REFERENCES

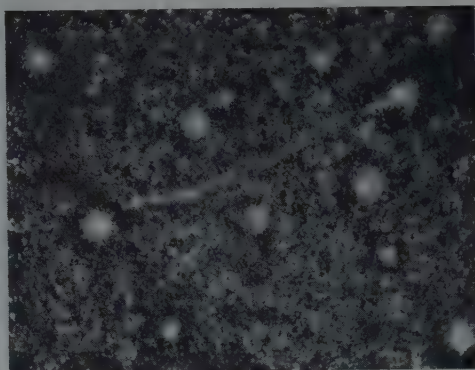
- AITCHISON, E. (1947).—Studies on the chromosomes of the Myrtaceae. *Amer. J. Bot.* **34**: 159, 407.
- ANDERSON, E. (1949).—"Introgressive Hybridization." (John Wiley & Son: New York.)
- BEASLEY, A. W. (1944).—Some notes on the Petrie Series, S. E. Queensland. *Proc. Roy. Soc. Qld.* **55**: 87–101.
- BLAKELY, W. F. (1934).—"A Key to the Eucalypts." (The Worker Trustees: Sydney.)
- BRETT, R. G. (1949).—*Rep. Aust. Ass. Adv. Sci.* **27**: 150.
- BROOKES, J. D. (1950).—Thesis, Melbourne University.
- CLAUSEN, J., KECK, D. D., and HEISEY, W. M. (1948).—Experimental studies on the nature of the species. III. Carnegie Inst. Wash. Publ. No. 581.
- CLIFFORD, H. T. (1953).—On the distribution of the species *Eucalyptus* in the region of the Dandenong Range, Victoria. *Proc. Roy. Soc. Vict.* **65**(I): 30–53.
- CLIFFORD, H. T. (1954).—A method for analysing hybrid swarms with special reference to *E. elaeophora* and *E. goniocalyx*. *Heredity* **8**: 259–69.
- DAY, W. R. (1951).—The susceptibility of strains of *Larix decidua* of varying geographic origins to injury by experimental freezing. *Forestry* **29**: 39–55.
- DUFFIELD, J. W., MIROV, N. T., and LIDDICOET, A. R. (1952).—Altitudinal races in ponderosa pine. *J. For.* **50**: 825–31.
- EWART, A. J. (1930).—"The Flora of Victoria." (Vict. Govt. Printer: Melbourne.)
- GREGOR, J. W. (1944).—The ecotype. *Biol. Rev.* **19**: 20–30.
- LEVITT, J. (1956).—"The Hardiness of Plants." (New York Academy Press.)
- MAIDEN, J. H. (1928).—"A Revision of the Genus *Eucalyptus*." (N.S.W. Govt. Printer: Sydney.)
- MCLAUGHLIN, I. (1956).—Report on *E. obliqua*. Botany Department, Melbourne University. (Mimeo.)
- PRYOR, L. D. (1951).—A genetic analysis of some *Eucalyptus* species. *Proc. Linn. Soc. N.S.W.* **76**: 140–48.
- PRYOR, L. D. (1953).—Genetic control in *Eucalyptus* distribution. *Proc. Linn. Soc. N.S.W.* **76**: 10–18.
- PRYOR, L. D. (1957).—Inheritance of some characters in *Eucalyptus*. *Proc. Linn. Soc. N.S.W.* **87**: 147–55.
- SMITH-WHITE, S. (1942).—Cytological studies in the Myrtaceae I. *Proc. Linn. Soc. N.S.W.* **67**: 335–42.

EUCALYPTUS REGNANS: THE SPECIES AND ITS FROST RESISTANCE

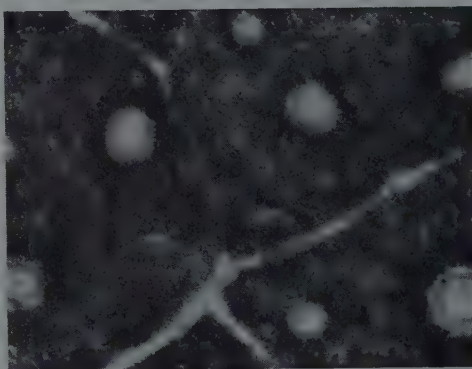
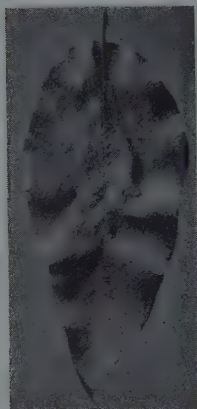
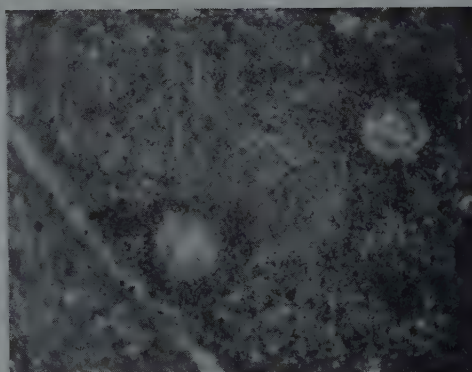


A fine stand of mature *Eucalyptus regnans* about 200 years old at Wallaby Creek, Vic.

EUCALYPTUS REGNANS: THE SPECIES AND ITS FROST RESISTANCE

E. regnans

Hybrid

*E. obliqua*

(a)

(b)

Leaves from the fifth node of seedlings used in this analysis. (a) $\frac{2}{3}$ natural size, showing texture of leaf surface. (b) Photomicrographs of whole mounts under low power ($\times 50$), showing oil glands from the adaxial surface.

EUCALYPTUS REGNANS: THE SPECIES AND ITS FROST RESISTANCE



A *Poa caespitosa* grassland frost pocket at 2050 ft, Wallaby Creek, showing the surrounding young stands of *E. regnans* and the marginal regeneration of *E. regnans* and *Acacia dealbata*.

TWO SPECIES OF SUBTERRANEAN CLOVER IN ISRAEL

By D. ZOHARY* and J. KATZNELSON*

[Manuscript received September 17, 1957]

Summary

Cytological and morphological study of subterranean clovers in Israel has shown that the previously reported $2n = 12$ Israeli "chromosomal race" or "cryptic species" is actually morphologically quite distinct from $2n = 16$ *Trifolium subterraneum* L.

Both on morphological and genetical grounds the "Israeli race", with chromosome number $2n = 12$, should be regarded as a distinct species. It is described in the present paper as a new species, *Trifolium israeliticum*.

The paper includes data on the distribution of the two subterranean clovers in Israel.

I. INTRODUCTION

Yates and Brittan (1952) reported on a new "chromosomal race" of *Trifolium subterraneum*, having a chromosome number of $2n = 12$, which they found in a collection obtained from Israel. Besides presenting cytological data, these workers (loc. cit.) as well as Brock (1953) reported on the failure to cross the new Israeli "race" to $2n = 16$ varieties of subterranean clover. Brock concluded that the new chromosomal race of $2n = 12$ should be regarded as a "cryptic species" within the *T. subterraneum* L. group.

In order to obtain additional information on speciation within the subterranean clovers, it was felt desirable to carry out a cytological and morphological survey of populations and forms of this group native to Israel. Work was started in spring 1956, when a few counts indicated to the present authors that the two "races" occur side by side at Beit-Qeshet, Lower Galilee. In spring 1957 a more extended survey was made, the results of which are presented in this report.

II. MATERIALS AND METHODS

Locations of subterranean clover from various parts of central and north Israel were selected for examination (see Fig. 1). These locations represent the major soil types and vegetation units in which subterranean clover is known to occur in Israel.

Fixations for chromosome counts from these locations were made in February and March 1957, at the beginning of the flowering season. The plants, or clusters of plants, from which fixations had been taken, were marked and visited again in March and April 1957 for collection of herbarium specimens and for the study of the morphology of well-developed plants.

Flower buds were fixed in 1:3 acetic-alcohol and were stored in this fluid, in a refrigerator, till their examination. Anthers from these flowers were squashed in 2 per cent. aceto-carmine. Chromosome number was counted in meiosis of microsporocytes,

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usually at Metaphase I, sometime also at Anaphase I. Chromosome determination, in each plant, was based on counts of at least five well-flattened pollen mother cells. In each station at least one plant, usually two, were examined.



Fig. 1.—Locations of *Trifolium subterraneum* and *T. israeliticum* examined in the present study.

●, *T. israeliticum*—chromosome number examined ($2n = 12$); ○, *T. israeliticum*—chromosomes not examined; ◆, *T. subterraneum*—chromosome number examined ($2n = 16$); ◇, *T. subterraneum*—chromosomes not examined.

III. CYTOLOGICAL OBSERVATIONS

Plants from 17 locations have been examined as to their chromosome numbers. Two chromosomal types were observed: one with $2n = 12$ and another with $2n = 16$. In their chromosome morphology these types were found to correspond to the figures given by Brock (1953). The results of the counts are given in Figure 1 and the description of the various locations from which chromosome counts were taken is given in Sections IV and V.

IV. MORPHOLOGICAL OBSERVATIONS AND TAXONOMICAL EVALUATION

Comparison of plants with chromosome number of $2n = 12$ with plants having $2n = 16$ chromosomes revealed the presence of sharp morphological differences between these two "chromosomal races" (see comparison below). Further field study of the populations from which counts were made, as well as examination of material from additional localities in Israel (see Fig. 1), showed that these morphological differences are constant. The two "chromosomal types" occurring in Israel were found to be easily separable from each other, on morphological grounds, both in the field and in herbarium material.

The presence of a distinct gap in the variation pattern, the difference in chromosome number, and the apparent existence of effective isolation barriers between the two forms—all these facts make it necessary to regard the two chromosomal types not as "cryptic species" but as two distinct, separate species. The $2n = 12$ "race" is therefore described here as a new species.

Trifolium israeliticum D. Zoh. & Katzn., sp. nov.; *T. subterraneum* L. var. *tel-avivensis* Eig in Second Contrib. Knowledge Flora Palest., p. 25, (1927).*

Annual, caulibus crassis, prostratis, 5–40 cm longis, fere a basi ramosis. *Folia* longe petiolata; foliola villosa, obovata, apice rotundato vel truncato, raro paulum emarginato, margine denticulato; stipulae ovato-oblongae. *Pedunculi* axillares, villosi, purpurascens, reflexi, petiolis plerumque crassiores, post anthesim excrecentes; capitula terrae adpressa vel terram ingredientia; capitulorum basium pedunculi breves, crassi, indurati incurvatique, petiolis plerumque bis crassiores. *Flores* fertiles corollati 2–5; corolla rubra usque ad violacea, 14–18 mm longa, calycis laciniis ter vel quater longior; calyx sparse villosus, calycis lacinae tubo paulum longiores; flores steriles numerosi, apetalii, post anthesim excrecentes, demum 2–5 capituli legumina obvolventes; calyces steriles stellatim expansi, laciniis plerumque hirsutis, irregulariter incurvatis. *Legumen* monospermum, exsertum, transverse ovatum, multo latius quam longum, coriaceum, induratum, margine superiori fortiter carinatum; legumen maturum transverse rugosum, nigro-violaceum usque ad purpureo-nigrum. *Semen* ovale, plerumque lutescens.

Annuals with thick, procumbent stems, 5–40 cm long. Number of basal runners in well developed plants usually 3–6, all branching almost from base of plant. *Leaves* with long petioles: leaflets obovate with rounded or truncated apex, rarely slightly emarginate, with denticulate margin, leaflets beset with long soft hairs; stipules ovate-oblong. *Peduncles* axillary, purplish, villous, reflexed, usually thicker than petioles, elongating after anthesis and burying the heads in the soil; lowest heads with short, thick, indurate, and incurved peduncles which are usually twice as thick as petioles. Corollate fertile *flowers* 2–5; corolla red to violet, 14–18 mm long, 3–4 times as long as calyx lobes; calyx lobes slightly longer than tube, sparsely hairy. Sterile flowers numerous, apetalous, growing after anthesis and finally enclosing the 2–5 pods of the head; sterile calyces, stellate-spreading, with irregularly bent and usually hairy lobes.

*Eig's description of *T. subterraneum* L. var. *tel-avivensis* is incomplete. It is based entirely on the corolla size and colour. However, his herbarium material agrees completely with *T. israeliticum*.

Pod exerted from split calyx, transversely oval, much broader than long, leathery, indurate, with strongly keeled upper margin, one-seeded; the surface of the ripe pod transversely wrinkled, dark violet to purplish black in colour. *Seed* oval, usually yellowish, tightly enclosed in pod. Chromosome number $2n = 12$.

Type locality.—Sharon Plain, Israel, between Pardes-Hanna and Karkur, altitude approx. 100 m. On top of red sandy loam hill with open herbaceous vegetation and few bushes of *Calycotome villosa*, D. Zohary Ts-701 (HUJ).

Type.—Hebrew University, Jerusalem. Cotype from type locality sent to the Herbarium, Royal Botanic Gardens, Kew, Great Britain.



Figs. 2-5.—Comparative drawings of the two subterranean clover species. Fig. 2.—Pod of *Trifolium israeliticum*. Fig. 3.—Pod of *T. subterraneum*. Fig. 4.—Seed of *T. israeliticum*. Fig. 5.—Seed of *T. subterraneum*.

Chromosome counts were also made in the following collections: LOWER GALILEE.—Beit-Qeshet, top of hill, patches of terra-rossa on somewhat soft Pliocene calcareous bedrock, hill sparsely covered with *Ceratonia siliqua* and *Pistacia lentiscus*, altitude 200 m, Katznelson Ts-702 (HUJ) $2n = 12$; about 200 m W. of Beit-Qeshet settlement, altitude 170 m, in patches of terra-rossa among hard dolomite rocks in heavily destroyed *Quercus ithaburensis* park forest, together with *Trifolium subterraneum*, Katznelson and D. Zohary Ts-705 and Ts-706 (HUJ) $2n = 12$; approx. 300 m W. of Beit-Qeshet settlement, in abandoned olive plantation with remnants of *Quercus ithaburensis*, terra-rossa soil on hard dolomite, Katznelson and D. Zohary Ts-707 (HUJ) $2n = 12$; about 400 m W. of Beit-Qeshet settlement, altitude 200 m, terra-rossa soil on hard crystalline limestone, park forest of *Quercus ithaburensis*, Katznelson Ts-708 (HUJ) $2n = 12$; about 500 m W. of Beit-Qeshet settlement, altitude 200 m, terra-rossa patches among hard limestone rocks in park of *Quercus ithaburensis* with *Carlina corymbosa* and *Echinops* sp., Katznelson Ts-709 (HUJ) $2n = 12$; approx. 4 km E. of Nazareth, 100 m W. of Sheikh el Ajami, altitude 500 m, in patches of deep terra-rossa soil on hard Eocene limestone hill, in association with *Carlina corymbosa* and *Echinops* sp., D. Zohary Ts-717 (HUJ) $2n = 12$. UPPER GALILEE.—Approx. 4 km N. of Safad, 30 m south of the road junction to Ein-Zeitim, altitude 700 m, in "pockets" of terra-rossa soil between hard limestone rocks, D. Zohary Ts-714 (HUJ) $2n = 12$; approx. 2 km W. of Jish, altitude 800 m, in patches of terra-rossa soil between hard limestone rocks, in association with *Poterium spinosum* and *Poa bulbosa*, D. Zohary Ts-721 (HUJ) $2n = 12$; about 1 km east of Kefar Biram, altitude 750 m, a small patch of meadow near road, in association with *Poa bulbosa* and *Lolium perenne*, terra-rossa soil on hard limestone bedrock, D. Zohary Ts-722 (HUJ) $2n = 12$.

Representative Specimens.—SHARON PLAIN.—Magdiel, 2. v. 1927, N. Naftolsky (HUJ). NORTH SAMARIA HILLS.—Approx. 2 km S. of Mishmar Haemeq, altitude 200 m, on top of basaltic hill in association with *Echinops* sp. and *Carlina corymbosa*, D. Zohary and Katznelson Ts-719 (HUJ).

Known Distribution.—Central and north Israel.

Trifolium israeliticum is characterized by its thick basal runners, short basal internodes, obovate leaflets, large red to violet flowers and its depressed hard pod. The main morphological differences between this head-burying species and *Trifolium subterraneum* L. in Israel can be summarized as follows:

T. israeliticum ($2n = 12$)

T. subterraneum ($2n = 16$)

- | | |
|---|---|
| (1) Leaflets usually obovate, with rounded or truncated apex. | (1) Leaflets usually deeply emarginate. |
| (2) Peduncles thick; the thickness is most obvious in lowest heads where the short, strongly incurved peduncles are usually twice as thick as petioles. | (2) Peduncles comparatively slender, equal in diameter to or thinner than corresponding petioles. |
| (3) Fertile flower with red or violet corolla, very large; corolla 14–18 mm long, 3–4 times as long as calyx lobes. | (3) Corolla of fertile flowers white, sometimes striped with pink; fertile flowers small, corolla length 8–14 mm, about twice as long as calyx lobes. |
| (4) Ripe pods hard, leathery, transversely oval, much broader than long, transversely wrinkled (Fig. 2), dark violet to purplish black, tightly enclosing the seed. | (4) Ripe pods crustaceous, obovate or broadly cuneate, longitudinally netted (Fig. 3), yellowish or brownish. |
| (5) Radicle tip situated at the narrow base of the seed (Fig. 4). | (5) Radicle tip bulges from the long side of the seed (Fig. 5). |

Apart from the abovementioned morphological differences, *Trifolium israeliticum* differs from *Trifolium subterraneum* forms, native to Israel, in being earlier in flowering and seed-setting. On the average, *T. israeliticum* plants were found to set fruit approximately 2–3 weeks earlier than *T. subterraneum*.

V. DISTRIBUTION OF TRIFOLIUM SUBTERRANEUM

In the course of the present study the following data were collected (see also Fig. 1) on the distribution of *T. subterraneum* L. in Israel.

Chromosome counts were made in the following collections: NORTH SAMARIA HILLS.—2 km S. of Mishmar Haemeq, altitude 200 m, on north-facing slope of basaltic hill, in association with *Echinops* sp. and *Carlina corymbosa*, *D. Zohary* and *Katznelson* Ts-718 (HUJ) $2n = 16$; 1 km SW. of Dalia settlement approx. 10 km east of Zichron Yaaqov, altitude 200 m, in a small valley with deep rendzina soil and herbaceous cover of *Avena sterilis* and *Hordeum bulbosum*, *D. Zohary* Ts-725 (HUJ) $2n = 16$. LOWER GALILEE.—Approx. 400 m W. of Beit-Qeshet settlement, deep terra-rossa patches on hard dolomite bedrock, in park forest of *Quercus ithaburensis*, side by side with *Trifolium israeliticum* (patch was under cultivation prior to 1949), *Katznelson* and *D. Zohary* Ts-710 (HUJ) $2n = 16$; approx. 1500 m N. of Beit-Qeshet settlement, altitude 300 m, on terra-rossa soil on Eocene hard limestone bedrock, semi-steppe Batha formation with *Carlina corymbosa* and *Echinops* sp., *Katznelson* and *D. Zohary* Ts-711 (HUJ) $2n = 16$; 2 km E. of Beit-Qeshet, on east-facing slope of basalt terrace, altitude 150 m, semi-steppe Batha of *Echinops* sp. and *Carlina corymbosa*, *D. Zohary* and *Katznelson* Ts-713 (HUJ) $2n = 16$; approx. 600 m W. of Beit-Qeshet settlement, altitude 230 m, in patches of deep terra-rossa soil in *Quercus ithaburensis* park forest,

Katznelson Ts-715 (HJ) $2n = 16$. UPPER GALILEE.—Approx. 2 km E. of Nahf on Acre-Safad road, altitude 300 m, terra-rossa soil "pockets" among hard limestone rocks with sparse coverage of *Poterium spinosum* and together with *Trifolium israeliticum*, D. Zohary Ts-724 (HJ) $2n = 16$.

Representative specimens.—JUDEAN FOOTHILLS.—2 km S. of Tel el Gezer, approx. 10 km SE. of Ramle, altitude 200 m, in open Batha of *Poterium spinosum* and *Andropogon hirtus*, the clover plants growing in patches of somewhat deeper brown soil among hard limestone rocks, D. Zohary Ts-726 (HJ). SHARON PLAIN.—Magdiel, edge of small wadi, 23. iv. 1926, N. Naftolsky (HJ). LOWER GALILEE: Bnei-Berit settlement approx. 15 km E. of Afula, altitude approx. 0 m, on north-facing slopes near Jubla ruins, among basalt boulders, in association with *Echinops* sp. and *Carlina corymbosa*, D. Zohary and Katznelson Ts-720 (HJ).

VI. OTHER OBSERVATIONS AND COMMENTS

Trifolium subterraneum ($2n = 16$) and *T. israeliticum* ($2n = 12$) are both quite common in north and central Israel. In several places, especially in Galilee, these two species are important constituents of the natural range. Ecologically, it is hard at present to draw a sharp line of difference between the two forms. Both clovers occur in Israel on a wide variety of soils, such as red sandy loams, basaltic soils, terra-rossa and dark rendzinas. In several places the two species are found together. However, in general *T. israeliticum* was found to occupy somewhat drier habitats than *T. subterraneum*.

Another observation worth mentioning is the sympatric occurrence of these two clovers. Clusters or colonies of these two forms growing side by side or mixed populations of these species have been observed at Mishmar Haemeq in the north Samaria hills, at Nahf in Upper Galilee, and at Beit-Qeshet in Lower Galilee. At Beit-Qeshet this sympatric occurrence was studied in some detail and several clusters were checked as to their chromosome numbers. No intermediate forms were found in these places of contact and the absence of such intermediates can serve as an additional indication for the existence of effective reproductive barriers between these two forms in nature.

From the practical point of view, the agricultural development of *T. israeliticum* as a parallel species to *T. subterraneum* is quite promising. Its earliness of development and its occurrence in relatively drier places, even on calcareous bedrock, could be quite advantageous under Mediterranean conditions with early summer droughts.

VII. REFERENCES

- BROCK, R. D. (1953).—*Nature* **171**: 936.
YATES, J. J., and BRITTAN, N. H. L. (1952).—*Aust. J. Agric. Res.* **3**: 300.

THE GAMETOPHYTE AND EMBRYO OF *POLYPHLEBIUM VENOSUM* (R. BR.) COPELAND (HYMENOPHYLLACEAE)

By ILMA G. STONE*

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Summary

An account is given of the spore, its germination, the delicate filamentous prothallus, the reproductive organs, the embryo, and the young sporophyte of *Polyphlebium venosum*.

The characteristic germination, which is a modification of the four-celled or *Trichomanes* type, remains unchanged under various conditions. A rhizoid appears first, followed after several weeks by a filament cell. The symmetrical four-celled stage is not attained, but after 3 months another rhizoid or filament cell is often cut off from the primary cell.

The monoecious gametophyte is a branching uniseriate filament and bears numerous, small, stalked antheridia, each with a simple wall, and an operculum which is raised or shed to allow the spermatozoids to escape. The archegonia, with straight necks, and tiers of four to six neck cells, are borne on special structures, the archegoniophores.

The prothallus reproduces itself vegetatively by regeneration, and by gemmae which on germination produce rhizoids, filaments, and antheridia or more gemmae.

The embryo is the first described for the *Trichomanes* group, and the investigation showed it to be unusual. There is no primary root, a single archegoniophore may bear more than one embryo, and the axis of the embryo varies in its position relative to the neck of the archegonium, possibly because the archegonia are borne in various positions on the archegoniophore. The suggestion is made that the embryo develops in a manner which gives the foot as large a contact as possible with the source of food supply.

The gametophyte and embryo show some primitive characters, but the most conspicuous feature is specialization in the form of reduction as an adaptation to a moist and shady environment.

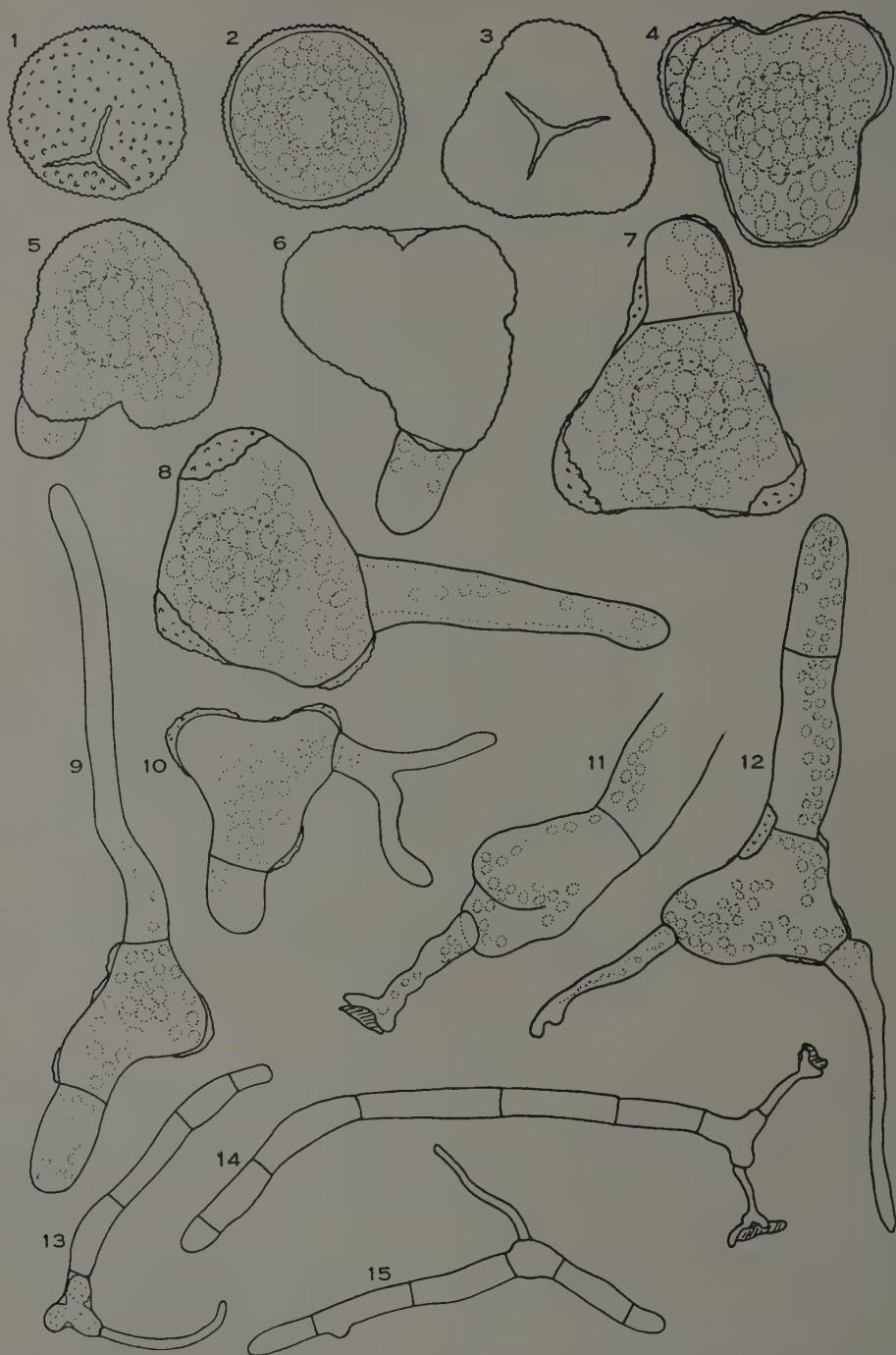
A key to the Victorian Hymenophyllaceae is appended.

I. INTRODUCTION

The Hymenophyllaceae formerly consisted of the three genera *Hymenophyllum* Smith, *Cardiomanes* Presl, and *Trichomanes* L., but Copeland (1933, 1937, 1938, 1947) reclassified the family and divided the species of the *Hymenophyllum* and *Trichomanes* groups into a number of genera, and *Trichomanes venosum* R.Br. was renamed *Polyphlebium venosum* (R.Br.) Copel., comb. nov. The genus *Polyphlebium* is monotypic and restricted in range to New Zealand, Tasmania, and eastern Australia. As the nomenclature has been changed a key is appended to facilitate field identification of the Hymenophyllaceae in Victoria.

The gametophytic generations of several species of the Hymenophyllaceae have been studied since 1843; the spores are comparatively easily germinated, but the

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Figs. 1-15.—Spore and early stages of gametophyte of *Polyphlebium*. Fig. 1.—Spore showing tetrad scar on proximal surface. $\times 790$. Fig. 2.—Spore, optical section. $\times 790$. Figs. 3, 4.—Gametophyte after 1 week on boiled water. $\times 790$. Figs. 5-8.—Fourteen days on boiled water. $\times 790$. Figs. 9, 10.—Six weeks on boiled water. $\times 400$. Figs. 11-15.—Three months on boiled *Dicksonia* roots. Figs. 11, 12 $\times 400$; Figs. 13-15 $\times 150$.

subsequent growth, in culture at least, is extremely slow. The most extensive work has been done by Holloway (1930, 1944) and Stokey (1940, 1948). Holloway grew two New Zealand species and also examined material from the field. He described the only embryo of this family which has been studied, that of *Cardiomanes reniforme* (Forst.) Presl, a genus considered to be closer to the *Hymenophyllum* than to the *Trichomanes* group. Stokey cultivated and studied 11 Javanese species, four of them belonging to the *Trichomanes* group, and in her papers reviews the earlier literature concerning germination and the vegetative and reproductive stages, showing that comparatively few species of the large *Trichomanes* group have been studied.

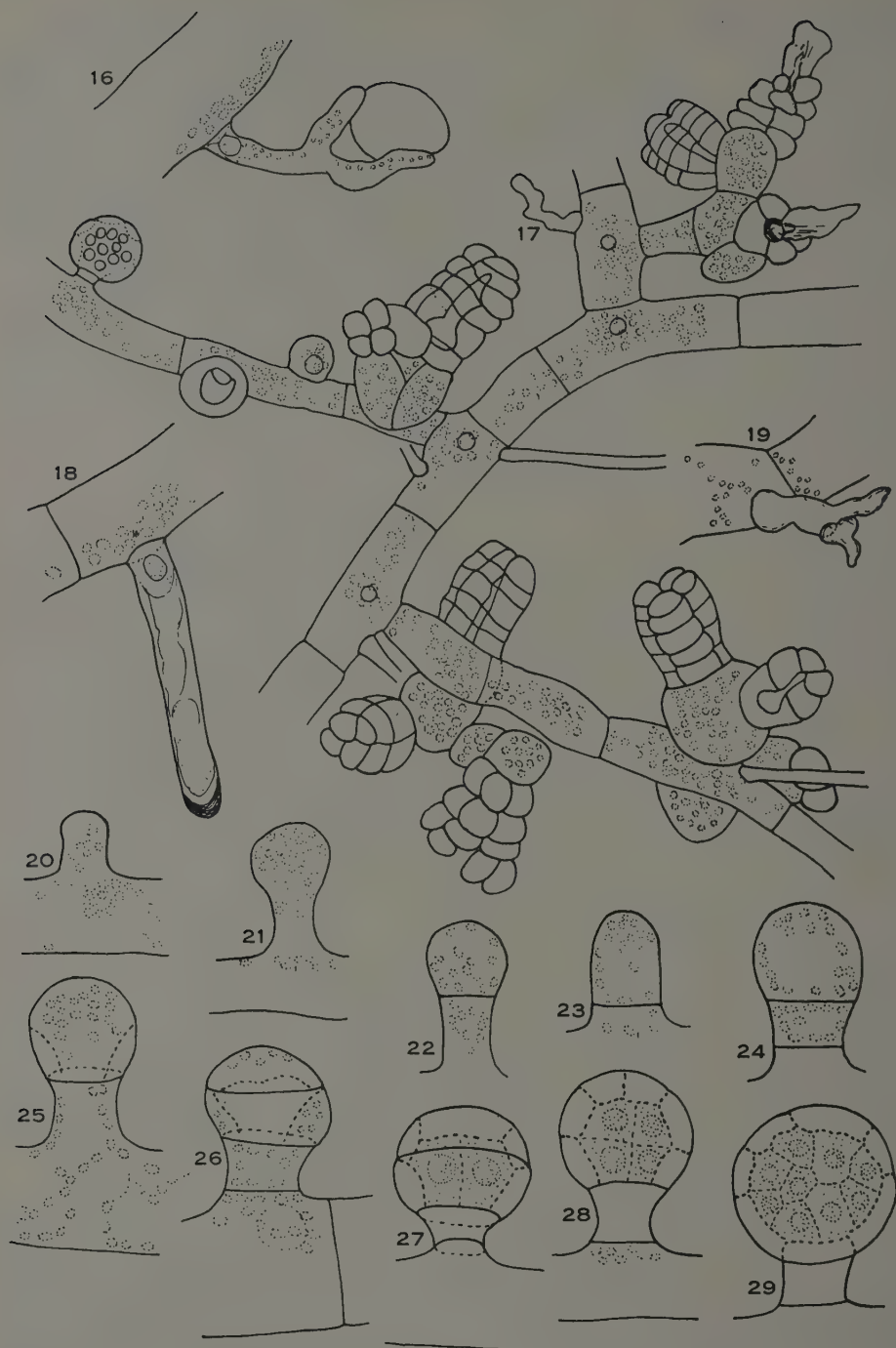
Goebel (1905) makes the only reference in the literature to the gametophyte of *Polyphlebium venosum*. He was sent material from the Black Spur, Marysville, Vic., and illustrates a portion of the filamentous gametophyte showing the formation of gemmae. Bower (1923) figures an antheridium of *Polyphlebium venosum* with 18 spermatocytes in optical section (from Goebel).

II. MATERIAL AND METHODS

Polyphlebium venosum is an epiphytic filmy fern growing almost exclusively on the trunks of *Dicksonia antarctica* Labill. (in Victoria). Mature gametophytes of *Polyphlebium* were collected in the forest near Melbourne in May, June, and July 1957. They were present in great abundance on the sides of the tree ferns with southern and eastern aspects, frequently on the under-side of leaning trunks, usually at the margins of mats formed by the sporophytes, but rarely where it was very wet. Little fertilization had taken place, probably because the autumn was unusually dry. In one instance they were growing on the humus in the old frond bases of a specimen of *Cyathea australis* (R.Br.) Domin, further up the side of the gully in a situation usually unfavourable for the growth of the mature sporophytes. Water would collect to a certain extent in these bases and it was chiefly from here that the embryos and young sporophytes were collected. Gemmae were also more prolific from this situation in May, but in later months, if conditions were moist, were found on almost all prothalli.

Material was fixed in chromacetic acid (chromic acid 1 g, glacial acetic 8 c.c., water 800 c.c.), embedded in paraffin, sectioned at 8–10 μ , and stained with Delafield's haematoxylin or Delafield's with safranin counterstain. Material was also preserved in a mixture of formaldehyde 5 c.c., glacial acetic acid 3 c.c., glycerin 20 c.c., and water 72 c.c. A 4 per cent. solution of potassium hydroxide was used to verify details of walls in antheridia. Camera lucida drawings were made of serial sections, and also of the external features from both fresh and preserved material.

In order to obtain the early stages in the development of the prothallus, sori bearing mature sporangia were sterilized by washing well under running water, then shaking for 10 min in a mixture of equal parts of a filtered solution of calcium hypochlorite (10 g : 150 c.c. water) and a 1 : 10 solution of "Savlon" (a wetting agent and bactericide) in sterile water. The sori were then washed in several changes of sterile water and the sporangia were allowed to dry and shed their spores, which were immediately sown on boiled water in sterile petri dishes. For later stages in development, similarly treated spores were sown on boiled *Dicksonia antarctica* and *Cyathea*



Figs. 16, 19.—Branched rhizoids. $\times 290$. Fig. 17.—Mature prothallus. $\times 190$. Fig. 18.—Rhizoid stained with iodine. $\times 440$. Figs. 20–29.—Optical sections showing development of antheridium. Figs. 27–29 show division of spermatogenous cell. Figs. 20–24 $\times 440$; Figs. 25–29 $\times 550$.

australis roots. The latter were found unsuitable as their surfaces dried quickly, unlike the *Dicksonia* roots with their numerous root hairs, which retained the moisture better. Cultures were found to prefer a shaded southerly window, stronger light being unfavourable.

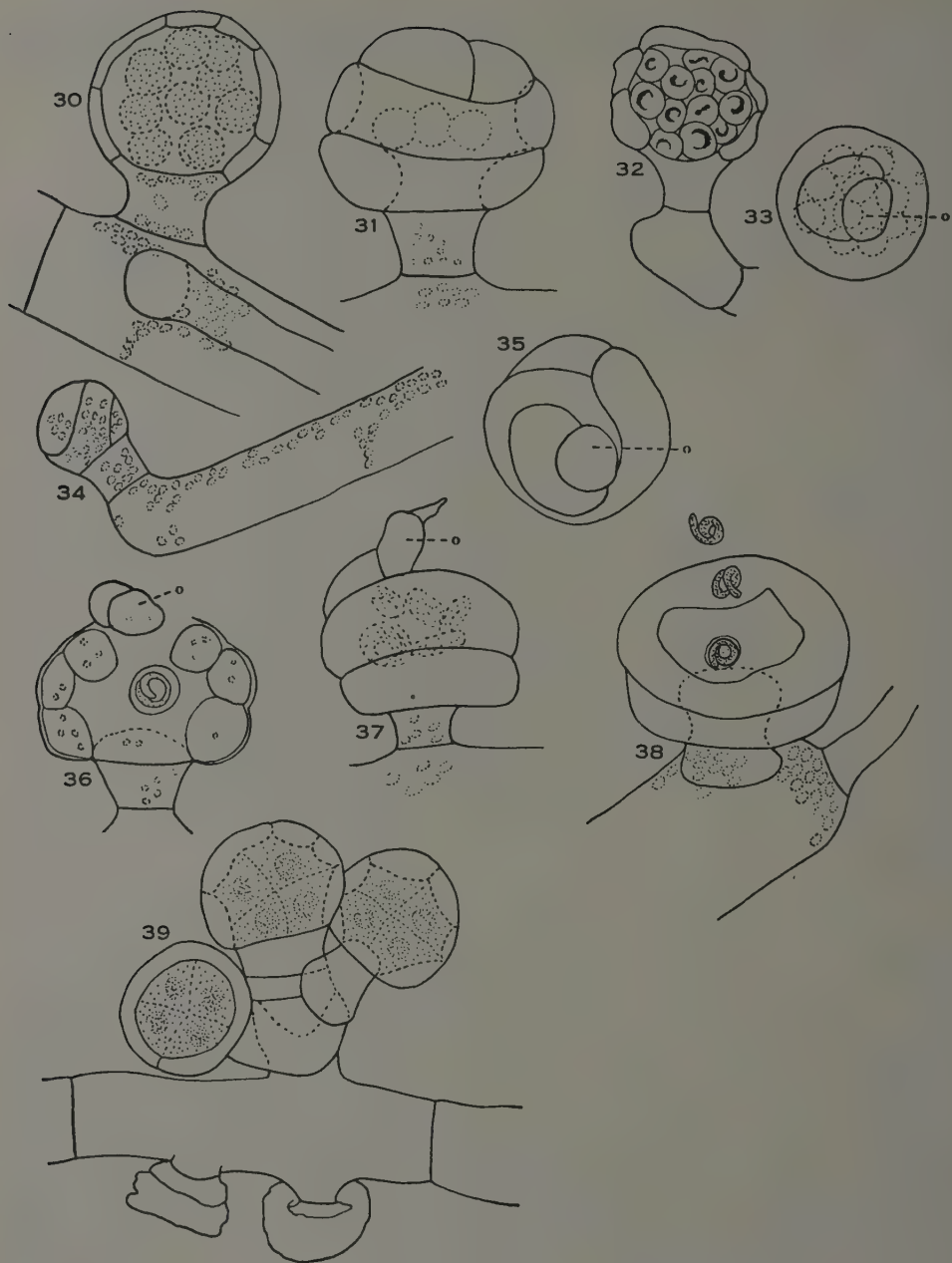
III. SPORE GERMINATION AND DEVELOPMENT OF THE PROTHALLUS

The terminology applied to the spore is that used by Harris (1955).

The dark green spores are subtriangular to globose in shape with a tetrad scar on the proximal surface, and the spore nucleus is visible as a lighter central region (Figs. 1, 2). The average diameter of the spores when shed naturally and mounted in water is 30 μ . The exine is thin and subgranulate and the triradial dehiscence fissures (laesurae) are long with narrow subgranular ridges. No septation was seen in the spore before or shortly after shedding, and during the first week on boiled water it became swollen and like a triangular cushion in shape, forming the primary prothallial cell (Fig. 3). At this stage the centre is very dense and dark green as the chloroplasts congregate around the nucleus, the tips of the triangular cell being paler in colour. The exine usually split along the laesurae, forming three valves, and the primary prothallial cell was orientated with its three tips towards the valves (Fig. 4). Figures 5–8 show the development after 14 days on boiled water. One tip of the primary cell extended and a rhizoid containing a few pale chloroplasts was cut off. This rhizoid elongated and became pale brown, and was followed at 6 weeks by a septation in the second tip, which produced the first cell of the filamentous prothallus (Figs. 9, 10). The third tip of the primary cell did not develop early, but cultures on tree fern roots examined after 3 months showed that it sometimes remained undeveloped, but usually produced a rhizoid, occasionally a filament (Figs. 11–15). After 5 months these cultures showed branching filaments of up to 20 cells with two or three rhizoids. Where conditions were crowded there appeared to be no difference in the type of germination.

In the cultures grown on water those prothalli which were floating produced unbranched rhizoids (Fig. 9), whereas in those which were submerged the rhizoids were branched, probably in response to contact with the glass (Fig. 10). The prothalli grown on tree fern roots produced short branched rhizoids, the branched portions being closely appressed to pieces of the tree fern and probably functioning as organs of attachment (Figs. 11, 14). Spores which had germinated in an indusium showed similar development to those in cultures, and branching rhizoids were occasionally found. From one locality in the field, when conditions were too moist for sporangia to dehisce, it was found that spores frequently germinated in the sporangium, the filaments projecting in all directions (but no spores which had just been shed were ever seen with any septations). No apospory was observed; it has been noted by Bower (1888) for other species of the *Trichomanes* group.

In its natural habitat the mature gametophyte consists of a branching, uniseriate filament, each branch usually arising from the distal end of a cell (Plate 1, Fig. 1). It grows amongst the root hairs of the *Dicksonia* tree fern, and bright green branches of the prothallus form a mat on the surface of the trunk. Occasional branching rhizoids occur, some with small chloroplasts (Figs. 16, 19) and many unbranched,



Figs. 30-33, 35-38.—Mature antheridia. *o*, Operculum. Fig. 30.—Optical section. $\times 550$. Fig. 31.—Ready to dehisce. $\times 550$. Fig. 32.—Section showing spermatozooids. $\times 440$. Fig. 33.—Top view showing operculum. $\times 440$. Fig. 34.—Young terminal antheridium, showing irregular development of wall. $\times 440$. Fig. 35.—Surface view from above, wall irregular. $\times 440$. Figs. 36-38.—Antheridia at dehiscence. $\times 550$. Figs. 36, 37.—Operculum not completely shed. Fig. 38.—Operculum cast off. Fig. 39.—Filament cell bearing several antheridia. $\times 400$.

either long or short, the tips frequently thickened and dark brown, but the walls paler further back (Fig. 18). Pale filament branches, with fewer chloroplasts, ramify between the root hairs and some bear numerous, long, thin, light brown rhizoids, frequently two or more to a cell. Also, amongst the root hairs, in close proximity to these pale branches, the archegoniophores are borne on cells usually very rich in chlorophyll, and often more robust than those of other parts of the prothallus. No longitudinal divisions of the filament were observed; they remain uniseriate as in the following species, which were all previously included in the genus *Trichomanes*: *Vandenboschia pyxidifera* (L.) Copel. (Bower 1888), *V. colensoi* (Hook.) Copel., *Macroglena stricta* (Menzies) Copel., and *Selenodesmium elongatum* (A. Cunn.) Copel. (Holloway 1930), *Vandenboschia maxima* (Bl.) Copel., and *Crepidomanes bilabiatum* (Nees & Bl.) Copel. (Stokey 1940). The cells are more slender and elongated than those of *Vandenboschia pyxidifera* (Bower 1888) in which they are not much longer than wide. In *Polyphlebium* the cells, although variable, are usually three and may be six or more times as long as wide. They appear more slender than those of *Crepidomanes bilabiatum* illustrated by Stokey (1948), for which no magnification was given.

The plants are monoecious, and antheridia were frequently found close to archegoniophores bearing archegonia (Fig. 17 and Plate 1, Figs. 1-3). Bower (1888) found that *Vandenboschia pyxidifera* was apparently dioecious, and Stokey (1948) observed that antheridia were scarce and rarely near archegonia in *V. auriculata* (Bl.) Copel., and possibly not on the same prothallus in *Crepidomanes bilabiatum*.

IV. REPRODUCTIVE ORGANS

(a) *Antheridia*

The antheridia were very numerous, frequently 10 or more green cells in a row bearing one or more antheridia and a rhizoid. Attempts to embed prothalli with attached antheridia were not very satisfactory as most of the antheridia became detached and lost during the washing process. However, being very small and simple, their structure could be seen in optical section and surface view, and they were observed at all stages of development.

The mature antheridia are small with a relatively simple wall, and contain a small number of spermatozoids, usually 10 showing in optical section. Each consists of a basal cell in the form of a delicate stalk, surmounted by a spherical head composed of a peripheral layer of four cells enclosing the spermatozoids (Figs. 30-32). They are usually intercalary in position (Plate 1, Fig. 2), but may be terminal (Figs. 34, 38).

The development of the antheridium is illustrated in Figures 20-29. The antheridium arises from a filament cell as an outgrowth which produces a spherical head either before or after it is cut off from the parent cell. The septum dividing the stalk cell from the filament is usually a little above the point of insertion on the filament, as in *Vandenboschia pyxidifera* (Bower 1888). The spherical head undergoes a series of divisions. A ring cell is cut off by an obliquely anticlinal wall, followed by a dome-shaped cell formed by a periclinal wall, thus producing a central spermatogenous cell. The dome-shaped cell divides to form another ring cell and the cap cell, the latter dividing to form an operculum which is raised or cast off when the antheridium dehisces (Figs. 36-38). The central cell, which is thus surrounded by a one-layered wall,

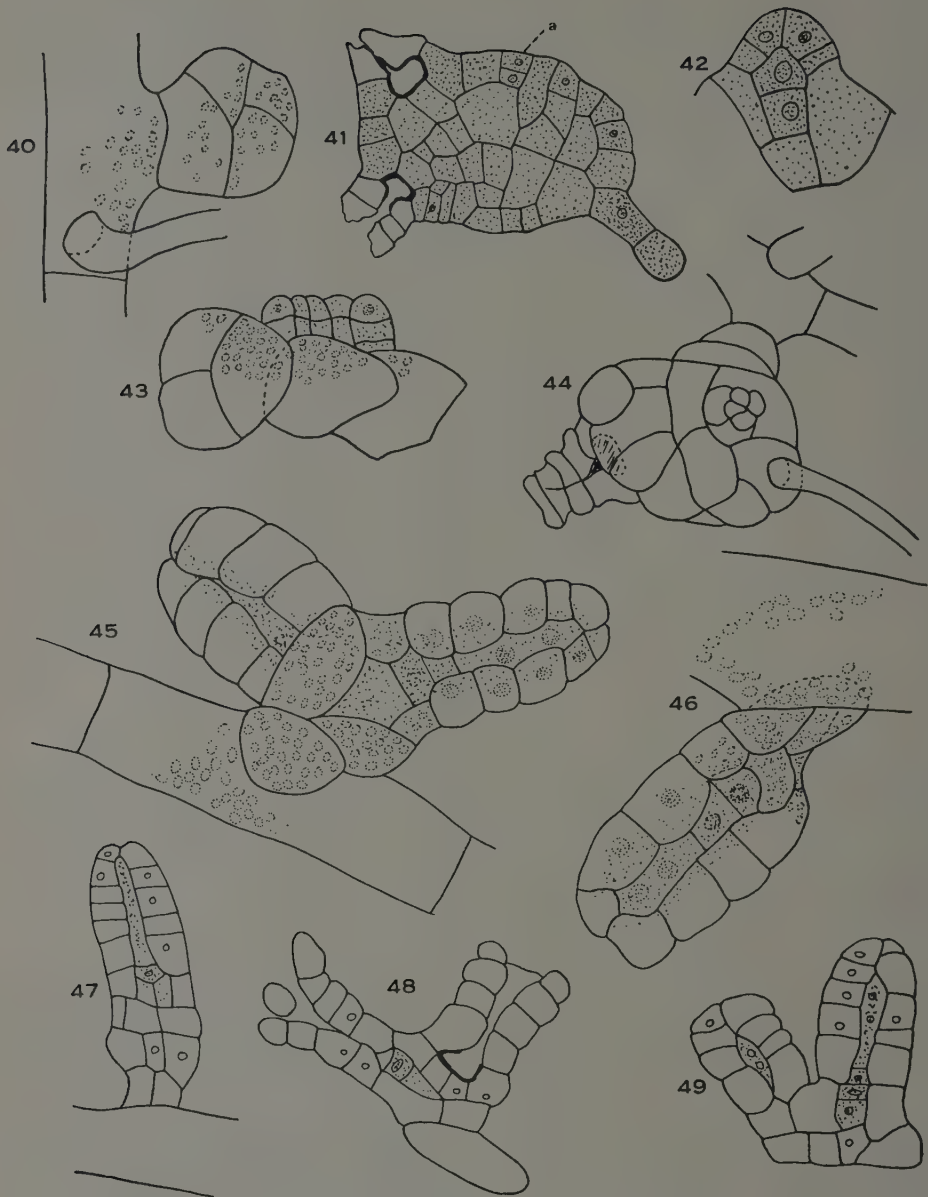


Fig. 40.—Young archegoniophore. $\times 440$. Fig. 41.—Section of large archegoniophore, showing first division of archegonial initial at *a*. $\times 170$. Fig. 42.—Section of young archegonium. $\times 440$. Fig. 43.—Surface view of young archegonia on part of archegoniophore. $\times 290$. Fig. 44.—Archegoniophore with rhizoid. $\times 290$. Fig. 45.—Optical section of small archegoniophore with two archegonia. $\times 360$. Fig. 46.—Similar, with one archegonium. $\times 360$. Figs. 47–49.—Sections of archegonia, showing variations in neck and basal cells. $\times 290$.

undergoes a succession of divisions to produce the spermatozoids (Figs. 27–30). In May and June and again in December antheridia were seen discharging sperms, which escaped from their membranes and swam away. Frequently the operculum was not completely shed. The cuticle ruptured but the opercular cell was only partly freed, a hinge action being produced, and the sperms forcing their way past as they were expelled (Figs. 36, 37).

During development, the wall cells and particularly the stalk cell are very rich in chloroplasts and starch, but these practically disappear at maturity.

Occasional variations in development of wall structure were seen (Figs. 34, 35), and some large antheridia were found in which the size but not the number of wall cells was increased. They showed about 20 spermatozoids in optical section. On some prothalli in which antheridial production was very active, some cells produced more than one antheridium, two and three being frequent. Figure 39 shows a filament cell bearing two old antheridia which have dehisced and three young antheridia arising from a one-celled outgrowth. Copeland (1938) when discussing the peculiarities of hymenophyllaceous gametophytes says that "Goebel reports countless lateral antheridiophore outgrowths from the ribbon-like prothallus of *T. (Didymoglossum) Kraussii*", but to date no such growths have been reported for a filamentous type of prothallus.

(b) *Archegonia*

The archegonia are borne on special structures, the archegoniophores, which usually arise from an intercalary cell of a filament (Fig. 17). The archegoniophores are stalked or sessile and may bear rhizoids (Figs. 41, 44, 45), and at first appear to have an apical cell (Fig. 40) which is soon lost, and they become a mass of cells of varying size and shape. Large ones bore up to 20 archegonia of various ages; in many cases, however, they were borne singly or in pairs on greatly reduced archegoniophores consisting of only a few cells which were probably produced when optimum conditions for growth had passed (Figs. 45, 46). Mettenius (1864) reports a reduced archegoniophore with two archegonia from an undetermined species. Figure 41 shows a section of a large archegoniophore attached by a stalk to the filament cell seen in cross section. It shows two old archegonia and the probable first division of an archegonial initial to form an outer primary neck cell and an inner cell. The next stage, when the inner cell divides to form a basal and a central cell, was not seen. Figure 42 shows a section of an archegonium at the stage when the neck cell has divided into four and started to produce rows of neck cells, the central cell pushing up between them. A surface view of developing archegonia on portion of an archegoniophore is shown in Figure 43. Figure 46 is an optical section of an archegonium which projects strongly from a very small archegoniophore. There are only three neck cells in each tier and they have probably not completed their divisions. The ovum, ventral canal cell, and neck canal cell with two nuclei can be seen (as in typical leptosporangiate ferns). The neck cells are finally in tiers of four, five, or six cells each; the number and size of cells in a row may vary but the neck remains approximately straight as in the Osmundaceae (Figs. 45, 47–49). Variations may occur in the basal cell, which may divide longitudinally (Fig. 47), or may be pointed (Fig. 48), perhaps indicating that the archegonial initial was pyramidal in shape as Stokey (1948) reports for *Vanden-*

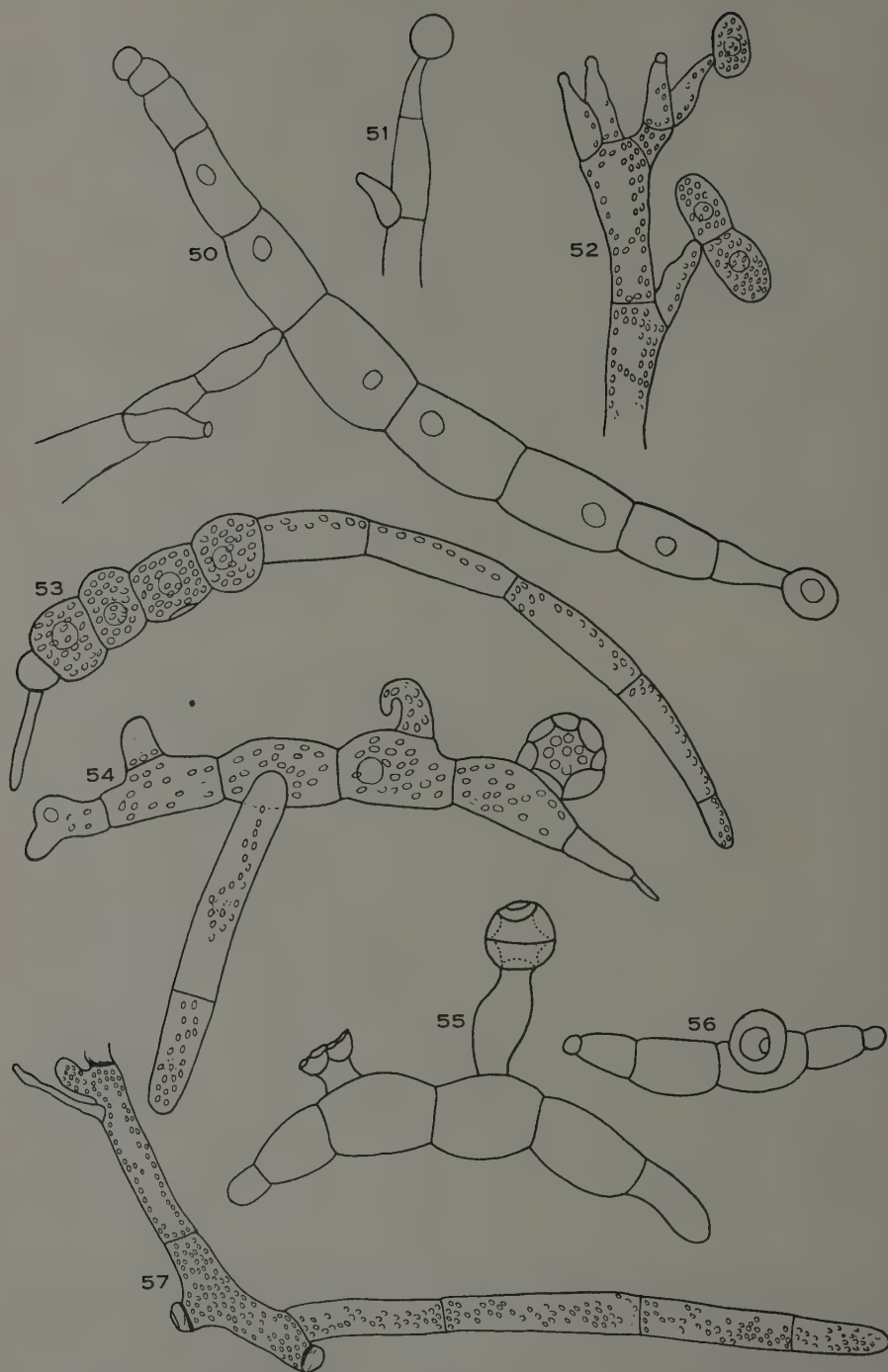


Fig. 50.—Large gemma still attached to sterigmata. $\times 190$. Figs. 51, 52.—Development of gemmae on sterigmata. $\times 190$. Figs. 53–56.—Germinating gemmae. $\times 190$. Figs. 54–56.—Gemmae bearing antheridia. Fig. 57.—Small piece of prothallus, showing regeneration. $\times 170$.

boschia auriculata and *Crepidomanes bilabiatum*. The jacket cells of the venter are not well developed when the archegonium is mature, but after fertilization form a well-defined calyptra to protect the developing embryo.

V. VEGETATIVE REPRODUCTION

(a) *Regeneration*

As is common in other members of the Hymenophyllaceae, regeneration of the prothallus occurs, and small pieces of old prothalli showing necrosis produced fresh filaments in the spring (Fig. 57).

(b) *Gemmae*

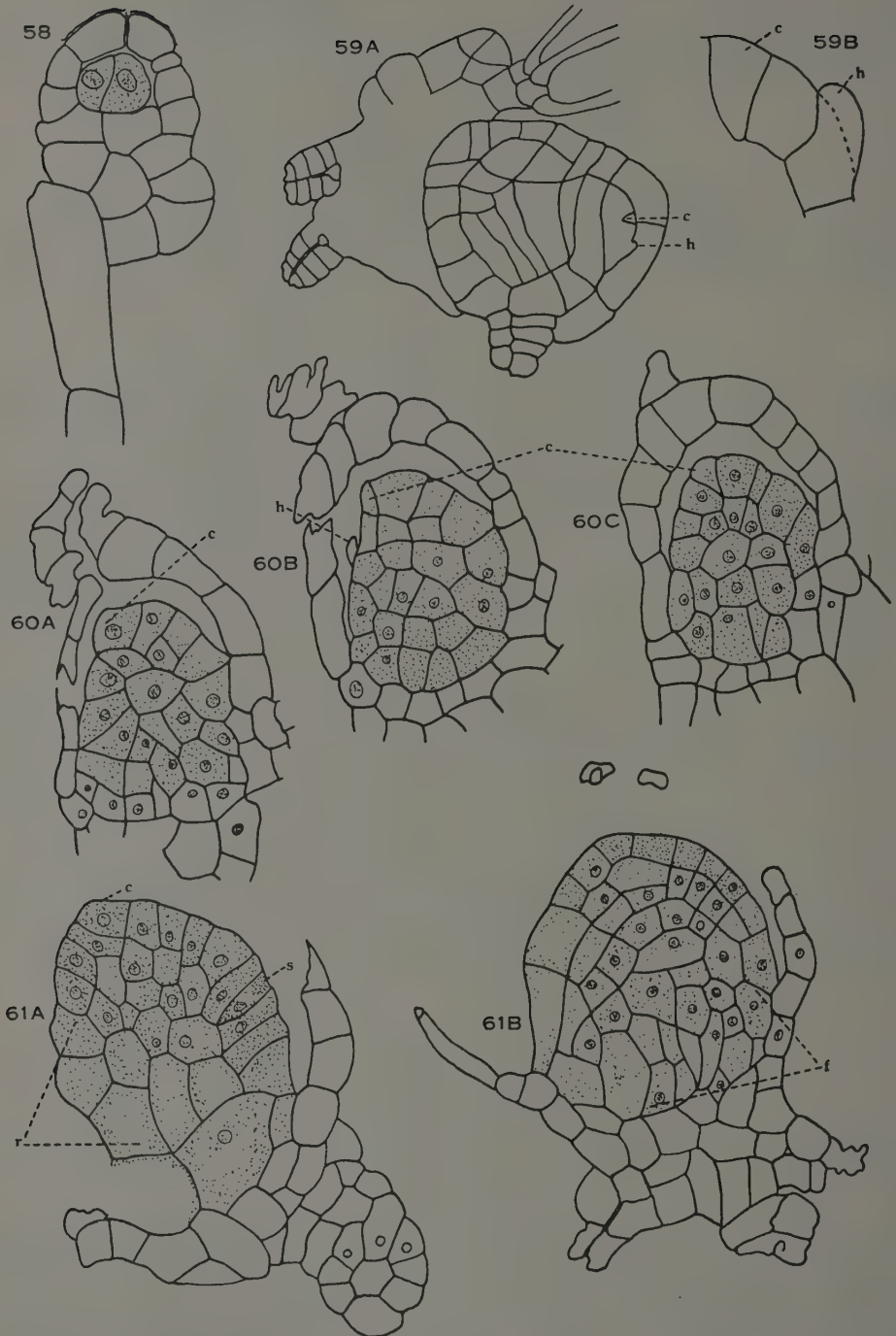
Vegetative reproduction of the prothallus by gemmae, illustrated by Goebel (1905), was most prolific in the material collected from *Cyathea australis* during May, but in the late winter all the gametophytes observed in the field and those growing in the laboratory were producing gemmae, while production of archegonia and antheridia ceased. Gemmae also developed in great profusion on prothalli transplanted on to boiled *Dicksonia* roots and kept in a humid atmosphere. Gemmae planted on *Dicksonia* roots in June germinated more quickly than spores, and produced antheridia directly and branching filaments bearing antheridia, but no archegoniophores by December, possibly because the gametophytes were not large enough.

The gemmae were examined at all stages from their formation on pale sterigmata projecting from terminal or intercalary cells of the filament to maturity and germination (Figs. 50–56). The mature, spindle-shaped gemmae may be from 2 to 12 cells in length, deep green, and packed with chloroplasts, oil, and starch, the two end cells usually being paler and smaller. On germination each produces a pale green germ tube which develops to a branching filament. Rhizoids or filaments may be produced by any cell of a gemma (Figs. 53, 54), and the branching filaments may bear antheridia or sterigmata and more gemmae. Antheridia may also arise directly from a cell of a gemma (Figs. 54–56), as reported by Georgevitch (1910) for *Trichomanes kaulfussii* (now *Macroglena setacea* (v. d. B.) Copel.).

VI. EMBRYO AND YOUNG SPORELING

No embryo of the *Trichomanes* group has previously been described, and a study of the *Polyphlebium* embryo revealed that it is not typical in form, the most remarkable feature being that the root primordium remains in a rudimentary condition so that no primary root develops.

A large number of embryos of *Polyphlebium* were examined and sectioned, but the direction in which sections were cut was a matter of chance, as archegonia are arranged in an irregular manner on an archegoniophore. Figure 58 shows a slightly oblique section of the only two-celled embryo which was cut, and in this the basal or first wall was apparently parallel with the axis of the archegonium, as is normal for leptosporangiate ferns. The cells surrounding the dividing zygote stain deeply, especially the lowest cells of the neck tiers. This is probably due to mucilaginous



Figs. 58–61.—Embryos of *Polyphlebium*. *c* Cotyledon initial; *h*, hair; *s*, stem initial; *r*, root primordial region; *f*, foot. Fig. 58.—Slightly oblique longitudinal section of a two-celled zygote. $\times 290$. Fig. 59*A, B*.—*A*, surface view of young embryo enclosed in calyptra. The

[For continuation see opposite page.]

contents. The figure also shows the filamentous prothallus attached to the archegoniophore.

The next stages of segmentation were not seen but Figures 60*A-C*, which are three consecutive sections of a young embryo, show that the cotyledon takes precedence early and is the most conspicuous feature of the embryo, its apical cell being



Fig. 62*A-C*.—Three sections of an older embryo, *B* and *C* consecutive. Small rhizoids are present in the root primordial region, the cotyledon initial is seen in *A*, and rudimentary vascular tissue between the cotyledon and root primordial region in *B* and *C*. $\times 190$.

the only initial which could be determined with certainty at this stage. The cotyledon initial, near which a hair is developing, is directed towards the remains of the archegonial neck which can be seen on the calyptra which surrounds the embryo. The calyptra, distended by the growth of the embryo, appears to rupture first below the archegonial neck on the side where there are several long, thin-walled transparent cells (also seen in Fig. 59*A*). The majority of the cells of the calyptra, and particularly

Figs. 58–61 (Continued)

archegoniophore has three rhizoids. $\times 150$. *B*, enlarged view of hair near cotyledon initial in *A*. $\times 440$. Fig. 60*A-C*.—Three consecutive sections of an embryo, showing the cotyledon initial, and calyptra with remains of archegonial neck. $\times 290$. Fig. 61*A, B*.—Two non-consecutive sections of an older embryo which has burst through the calyptra. *A* shows cotyledon and stem initials and possible root primordial region. $\times 290$. *B* shows foot. $\times 210$.

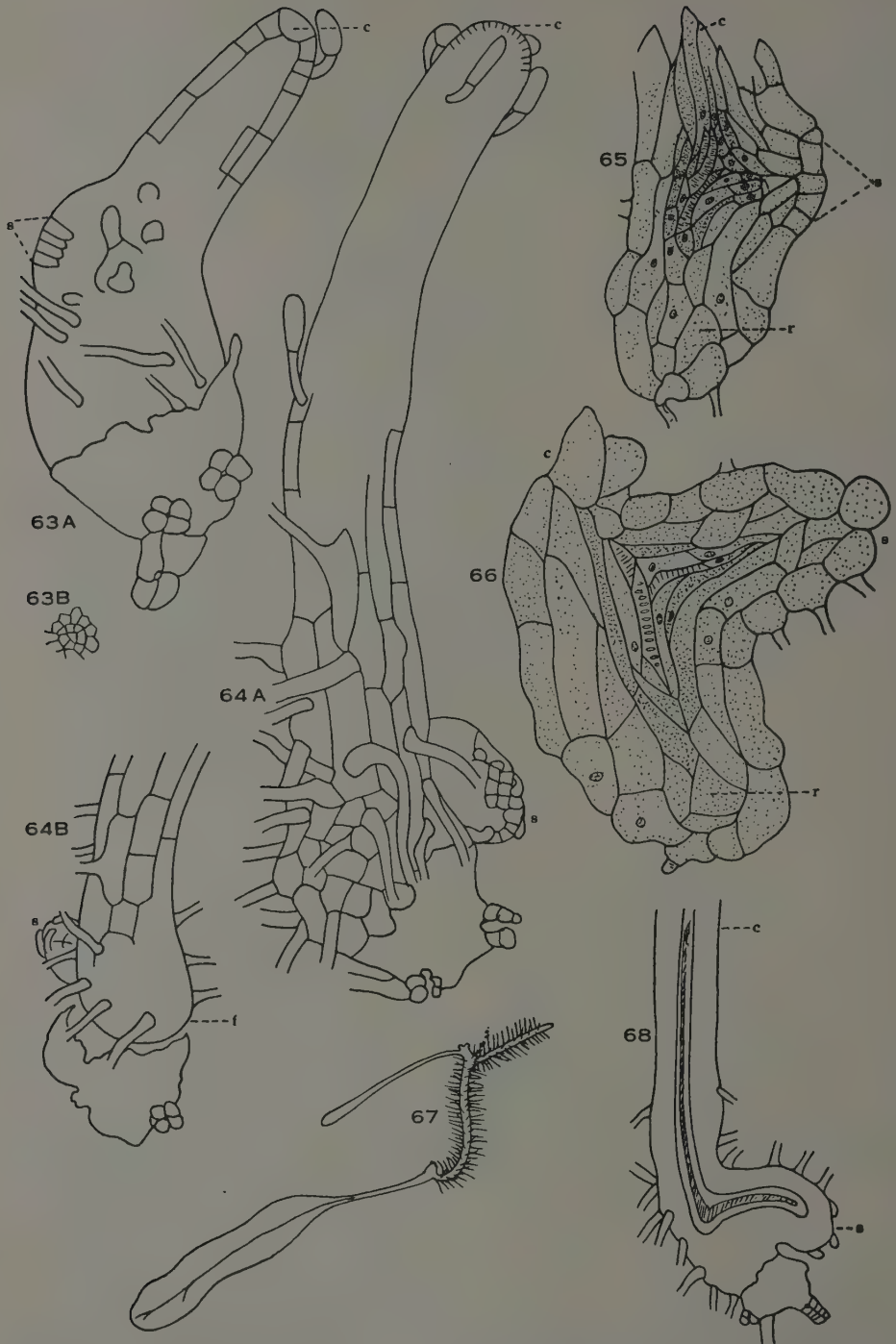


Fig. 63A, B.—External view of a well-developed embryo attached to an archegoniophore. The cotyledon initial, glandular hair, flat stem apex, and rhizoids are indicated in A. B shows stem apex in surface view. $\times 110$. Fig. 64A, B.—Similar, of young sporophyte. A shows marginal

[For continuation see opposite page.]

those at the base of the archegonial neck, stain deeply and probably have mucilaginous contents. The archegoniophore which bore this embryo also showed a fertilized zygote arrested in its development, another partly grown and shrivelled embryo facing in the opposite direction from the one figured, and a well-developed embryo with an advanced cotyledon growing out in a direction almost at right angles to the embryo figured.

Figure 59*A* shows an external view of an embryo enclosed in its calyptra, which is transparent in the region of the cotyledon. The cotyledon initial in this embryo is not directed towards the archegonial neck, so the axis of the embryo may vary in its relation to the axis of the archegonium. A small hair which is forming near the cotyledon initial is shown at higher magnification in Figure 59*B*. This later becomes a two-celled glandular hair and probably protects the apical cell from desiccation when it is no longer protected by the calyptra.

An older embryo is shown in Figures 61*A* and 61*B*. These longitudinal sections are not consecutive. Figure 61*A* shows the apical cells of the cotyledon and stem. The initial of the stem is deep and narrow in contrast to that of the cotyledon, which is broad and conical. The root primordium could not be determined with certainty, but may be in the region indicated on the figure. The foot can be seen in Figure 61*B*.

Figures 62*A–C* show longitudinal sections of a more advanced embryo (62*B* and 62*C* are consecutive) in which the cotyledon has elongated and has developed three glandular hairs. Rudimentary vascular tissue has developed between the cotyledon and the primordial region of the root, and at a later stage the vascular tissue from the stem joins into this. Small rhizoid-like hairs are developing from the epidermal cells in the area considered to be the root region. These hairs are cut off by a septum from the epidermal cells and become brown in colour. Copeland (1938) uses the term "rhizoid" for the fine hairs on the rhizomes of epiphytic filmy ferns, and that term is used here for the rhizoid-like hairs on embryo and rhizome.

The foot, which projects strongly from the archegoniophore, can be seen beside the root region. The epidermal and hypodermal cells in the region of the foot and root primordium are large and later become filled with tannin. The suppression of the root gives the embryo a finger-like appearance, quite unlike the typical leptosporangiate embryo, which is prone at this stage.

On the same archegoniophore another embryo, which was cut through in a direction perpendicular to the axis of its parent archegonium, was orientated at right angles to the one described.

As is usual in leptosporangiate ferns, the stem area is late in developing and the stage at which the stem appears is probably controlled by external conditions.

Figs. 63–68 (*Continued*)

meristem of the cotyledon, several glandular hairs, rhizoids, and the developing rhizome with hairs at the apex. $\times 110$. In *B*, the archegoniophore has been removed to expose the smooth green foot. $\times 80$. Fig. 65.—Section of young sporophyte, cotyledon and stem apex oblique. $\times 140$. Fig. 66.—Similar section of older sporophyte. $\times 140$. Fig. 67.—Sporophyte with branching rhizome covered with rhizoids, and second leaf. $\times 5$. Fig. 68.—Optical section of young sporophyte, showing outline of vascular tissue. $\times 80$.

Sometimes it appears while the cotyledon is still a cylindrical organ but it may be delayed until the lamina wings are formed. Figure 63A is an external view of a well-grown embryo, showing the apical region of the stem which is just beginning to advance. Figure 63B is a surface view of the stem initial, which is triangular in outline. The stem initial is narrow and deep and the stem apex flattened as Bower (1889-90) described for the rhizome of the mature sporophyte of *Trichomanes radicans* (now *Vandenboschia radicans* (Sw.) Copel.), a more robust species. Numerous rhizoids have developed from the tannin-filled epidermal cells at the proximal end of the embryo and a few have formed on the lower part of the cotyledon. They become brown and probably function as organs of fixation and may also protect the embryo from desiccation. The cotyledon is still cylindrical with a broad apical cell.

In Figure 64A the cotyledon is almost 1 mm long and the apical cell has been replaced by a marginal meristem, and narrow one-layered lamina wings have developed. The cotyledon does not show circinate vernation. Two-celled glandular hairs, the narrow basal cells of which contain tannin, protect the meristem and also develop along the midrib. The apex of the stem, or rhizome, is deep green and protected by several glandular hairs.

Figure 64B shows the same embryo with the archegoniophore and remains of the calyptra removed to expose the small deep green foot.

Figures 65 and 66 are sections of young sporelings showing the probable root primordial region. The section of cotyledon and stem is oblique, but it passes through the junction of the vascular tissue where the tracheids are shorter and thicker and shows the short extension of the latter towards the root primordium. The embryo in Figure 66 is more advanced in growth of cotyledon and rhizome, but shows that no increase in cells has occurred in the root region.

The outline of the vascular system with the short stump which extends towards the suppressed root can be seen in the optical section of a young sporeling shown in Figure 68.

Figure 67 pictures a young sporophyte in which a second linear leaf has arisen from the creeping rhizome. The first leaf has lateral veins and the rhizome is branched and covered with numerous rhizoids, but no roots have developed by this stage.

VII. DISCUSSION

The Hymenophyllaceae is considered to be a relatively primitive leptosporangiate family, further advanced than the Osmundaceae but not as highly evolved as the Polypodiaceae, and probably parallel in development with the Gleicheniaceae and Schizaeaceae. In the sporophyte, specialization in the form of reduction has taken place, the leaf being simplified as an adaptation to its environment of high humidity and weak light. A parallel development has occurred in the gametophytic generation, which has become small and delicate. The *Hymenophyllum* group is regarded as more primitive than the *Trichomanes* group, with *Cardiomanes* closer to the former. In general the *Hymenophyllum* group has a ribbon-like prothallus with discontinuous marginal cushions, while the *Trichomanes* group has a filamentous

prothallus with discontinuous archegoniophores, believed to be derived by reduction from the ribbon type. Copeland (1933, 1937, 1938) considers that *Polyphlebium*, a genus of restricted distribution following a line of development of its own, has closest affinities with the genus *Vandenboschia*, which he describes as the most primitive and the most nearly cosmopolitan of the *Trichomanes* group.

Three types of spore germination have been described in the family:

- (1) The *Hymenophyllum* type, in which two primary walls produce a triradiate division into three cells, this often occurring before the spore is expelled from the sporangium. These cells enlarge and the tip of each is cut off by a secondary wall, each distal cell forming a rhizoid or a short filament.
- (2) The *Trichomanes* or four-celled type, in which three cells are cut off at the tips of the triangular swollen spore leaving a larger central cell. Stokey (1940) demonstrated how the four-celled type believed typical of the *Trichomanes* group could have evolved from the three-celled *Hymenophyllum* type by the suppression of the two primary walls.
- (3) In some species there is a modification of the four-celled type in which the symmetrical appearance is not attained because of delay or suppression in the formation of walls in one or two of the tips.

Polyphlebium exhibits this third kind of germination, which is considered to be the furthest removed from the central *Hymenophyllum* type. Germination for about nine species of the *Trichomanes* group has been described, six of these being in the genus *Vandenboschia*. Three species of *Vandenboschia* have the modified four-celled type and this has not previously been described for any other genus. However, of the other three species of *Vandenboschia* described, one has the more primitive three-celled type, and two have the symmetrical four-celled type.

Polyphlebium has a purely filamentous gametophyte which though delicate is long-lived, and in this resembles the more primitive leptosporangiate ferns. The mature gametophytes of *Vandenboschia pyxidifera* (Bower 1888), *V. colensoi* (Holloway 1930), and *V. maxima* (Stokey 1948) have been described as filamentous, but *V. auriculata* (Stokey 1948) has some flattened expansions.

The antheridia in the *Hymenophyllum* group are of a primitive type, large and complicated in wall structure and with a large sperm output. Those of the *Trichomanes* group are smaller and simpler and with a smaller sperm output. As Stokey (1948) says, although this may indicate a derived group it is also to be expected from a smaller, simpler gametophyte. *Polyphlebium* has a small antheridium but produces a great number of antheridia, and the sperm output per prothallus must be extremely high. Stokey reported very few antheridia on prothalli of *Vandenboschia auriculata* and *Crepidomanes bilabiatum*. The antheridium of *Polyphlebium* resembles that illustrated by Bower (1888) for *Vandenboschia pyxidifera* in having a more definite pedicel than those of *Crepidomanes bilabiatum*, *Vandenboschia maxima*, and *V. auriculata*, which have a disc or wedge-shaped basal cell (Stokey 1948). The number of sperms in optical section, however, was usually 10–12, which would be closer to those of *V. auriculata* and *V. maxima* than to *V. pyxidifera* with 20 in optical section.

The archegonium of the *Hymenophyllum* group has a long straight neck, which is usually associated with primitive ferns, whereas all the descriptions of the arche-

gonium of the *Trichomanes* group picture it with a short straight neck of four cells or less in each tier, except that of *V. pyxidifera*, which usually has five but may have up to seven. The archegonium of *Polyphlebium* frequently has more than four cells in each tier and in this respect resembles those of *V. pyxidifera*. It resembles the archegonium described for the Hymenophyllaceae by Stokey (1948) in having an axial row normal for leptosporangiate ferns but with the ventral canal cell occupying less space in the venter and more in the neck than is usual, a basal cell which may be variously divided, and a poorly developed jacket layer, which is probably associated with the reduced nature of the prothallus but resembles that in the Marattiaceae.

The gametophyte of *Polyphlebium* probably shows closest resemblance to *Vandenboschia pyxidifera* and does not exhibit any characteristics by which it could be distinguished from *Vandenboschia* or *Crepidomanes*, the only two of Copeland's genera in the *Trichomanes* group with species which have been adequately described.

Bower (1923) sets out the comparative features of the embryo in the Filicales, the most important being the position of the basal wall and the relation of the body organs to it. In all ferns the epibasal hemisphere gives rise to the stem and cotyledon, the hypobasal to the foot, if present. The root may arise from either hemisphere, normally the hypobasal in leptosporangiate ferns. All delays or suppression in development of these organs are held as derived from the normal and described as biological adaptations, and precedence of one organ over another is related to nutrition.

In at least one case the position of the basal or first wall in the zygote of *Polyphlebium* was parallel to the long axis of the archegonial neck, which is normal for leptosporangiate ferns. However, this is possibly not always so, as the cotyledon which was directed towards the archegonial neck in several embryos studied was directed away from it in some cases, and in one embryo sectioned, the root region was directed towards the archegonial neck. Holloway (1944) found that in the embryo of *Cardiomanes reniforme*, the cotyledon and root apices had no constant relation to the archegonial neck and the basal wall was usually transverse or oblique, never truly vertical. He considered this to be remarkable, as a transverse basal wall is a characteristic of some eusporangiate ferns and is not present in the ancient leptosporangiate family Osmundaceae. He suggested that gravitational stimulus may have caused variations in the positions of the young embryos. In *Polyphlebium* the archegonia are arranged irregularly on the archegoniophores and the stimulus of gravity could possibly cause a change in the position of the basal wall in the zygote. However, a factor which negates this idea is that frequently more than one embryo developed on an archegoniophore, and in these cases the axis of the embryo was not constant in its relation to the axis of the parent archegonium, nor was there a constant relation in the orientation of one embryo to another. The main factor determining the position of the embryo may be the need to have the foot, which is the first absorbing organ, in contact with the greatest area of archegoniophore possible.

As in *Cardiomanes*, the organs of the *Polyphlebium* embryo appear to be differentiated later than they are in the more advanced leptosporangiate ferns and correspond more closely with the Osmundaceae. The appendages could not be assigned to any definite region of the two-celled embryo but on the evidence of older

embryos it is probable that the cotyledon and stem are produced from one half, the epibasal hemisphere, and the foot and the primordial root region from the other, the hypobasal hemisphere, as is normal for leptosporangiate ferns. Holloway (1944) could not determine this point definitely for *Cardiomanes* but considered that the cotyledon, root, and stem all originate from the epibasal cell and the foot from the hypobasal, more like the eusporangiate ferns.

The broad cotyledon initial appears first and the cotyledon takes precedence over the other organs to take over nutrition of the growing plant. There is an unusually early development of glandular hairs, which probably protect the cotyledon initial. Holloway observed an early development of hairs near the stem initial, but not the cotyledon in *Cardiomanes*.

The stem initial, which develops later, is narrower and deeper than is usual in higher leptosporangiate ferns and more like that described for the Osmundaceae by Cross (1931).

The most conspicuous feature of the *Polyphlebium* embryo is the absence of primary root, which is probably a biological adaptation to its hygrophytic habit. This suppression of the primary root is interesting as *Polyphlebium* at maturity is not a rootless form, although many of the epiphytic species in the *Trichomanes* group are without roots. In the genus *Vandenboschia* some of the species, including *V. pyxidifera*, which appears closely related to *Polyphlebium*, are rootless. It was not observed at what stage roots were formed by *Polyphlebium*, but they were not present in sporeling plants with two leaves and it seems that the filiform branching rhizomes with numerous rhizoids function largely in their place.

Goebel (1905) wondered if there would be a difference in the behaviour of the embryos of those species with and those without roots. Engler and Prantl (1902) illustrated a sporeling of *Trichomanes alatum* Sw. and one of *Trichomanes rigidum* (now *Selaginodesmium rigidum* (Sw.) Copel.) still attached to prothalli, and in each case there was a primary root. These two ferns belong to the *Trichomanes* group but are terrestrial. It may be that the epiphytic members of the group which have filiform rhizomes will be found to have rootless embryos. In *Cardiomanes* (Holloway 1944) the root develops equally with the cotyledon. The author has observed a well-developed primary root in two members of the *Hymenophyllum* group, *Hymenophyllum cupressiforme* Labill. and *Mecodium flabellatum* (Labill.) Copel. The only other fern which has been recorded as having a suppressed root in the embryo is *Salvinia natans* L., and this is interesting, as the heterosporous aquatic family Salviniaceae has important structural features in common with the Hymenophyllaceae and it has been considered that they are a highly specialized offshoot from the latter family.

The foot of the *Polyphlebium* embryo is small, as would be expected with such a delicate prothallus, but it is definitely haustorial in character, though not deeply embedded in the archegoniophore, and the embryo projects strongly. Holloway (1944) found that *Cardiomanes* had a large foot and the embryo projected strongly, and the author has observed a large round foot in the sporelings of both *Hymenophyllum cupressiforme* and *Mecodium flabellatum*.

Holloway also found that *Cardiomanes* was unusual in that the prothallus bore a plurality of embryos. This is also a feature of the *Polyphlebium* gametophyte, even a single archegoniophore often bearing more than one embryo.

The *Polyphlebium* embryo shows an early development of rhizoid-like hairs. They first arise from the epidermal cells in the root primordial region, but later from the foot and stem regions, and even extend to the lower part of the petiole of the cotyledon. They become brown like the epidermal cells in these regions. Cross (1931) reported that epidermal cells of the embryo of *Osmunda cinnamomea* L. became filled with tannin and oils in all regions except the actual meristems of leaf and stem, and those adjacent to the root apical became part of the root cap. He does not report rhizoids. The embryos of *Tmesipteris tannensis* Bernh. (Holloway 1917), which are rootless and have a similar habitat to those of *Polyphlebium*, also develop rhizoids at an early stage, and it seems probable that this is an adaptation to the epiphytic environment and possibly a safeguard against desiccation.

The embryo of *Polyphlebium*, like the gametophyte and the mature sporophyte, has some features more primitive than the advanced leptosporangiate ferns and shows specialization along the lines of reduction in adaptation to its moist and shady habitat; and this supports Bower (1926) in his opinion that the Hymenophyllaceae are a relatively primitive family exhibiting simplification.

VIII. ACKNOWLEDGMENTS

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IX. REFERENCES

- BOWER, F. O. (1888).—On the normal and abnormal development of the oophyte in *Trichomanes*. *Ann. Bot., Lond.* **1**: 269–305.
- BOWER, F. O. (1889–90).—A comparative examination of meristems of ferns as a phylogenetic study. *Ann. Bot., Lond.* **3**: 305–92.
- BOWER, F. O. (1923).—"The Ferns." Vol. 1. (Cambridge Univ. Press.)
- BOWER, F. O. (1926).—"The Ferns." Vol. 2. (Cambridge Univ. Press.)
- COPELAND, E. B. (1933).—*Trichomanes*. *Philipp. J. Sci.* **51**: 119–280.
- COPELAND, E. B. (1937).—*Hymenophyllum*. *Philipp. J. Sci.* **64**: 1–188.
- COPELAND, E. B. (1938).—Genera Hymenophyllacearum. *Philipp. J. Sci.* **67**: 1–110.
- COPELAND, E. B. (1947).—"Genera Filicum." (*Chronica Botanica*: Waltham, Mass.)
- CROSS, G. (1931).—The embryology of *Osmunda cinnamomea*. *Bot. Gaz., Lond.* **92**: 210–17.
- ENGLER, A., and PRANTL, K. (1902).—"Naturliche Pflanzenfamilien." 1 Teil, Abt. 4. (Verlag von Engelmann: Leipzig.)
- GEORGEVITCH, P. (1910).—Preliminary note on apospory and apogamy in *Trichomanes kaulfussii*. *Ann. Bot., Lond.* **24**: 232–4.
- GOEBEL, K. (1905).—"Organography of Plants." Vol. 2. 2nd English Ed. Trans. I. B. Balfour. (Oxford.)
- HARRIS, W. F. (1955).—"A Manual of the Spores of New Zealand Pteridophyta." (N.Z. Dep. Sci. Industr. Res.: Wellington.)
- HOLLOWAY, J. E. (1917).—The prothallus and young plant of *Tmesipteris*. *Trans. Proc. N.Z. Inst.* **50**: 1–44.
- HOLLOWAY, J. E. (1930).—The experimental cultivation of the gametophytes of *Hymenophyllum pulcherrimum* Col. and *Trichomanes reniforme* Forst. *Ann. Bot., Lond.* **44**: 269–84.

GAMETOPHYTE AND EMBRYO OF *P. VENOSUM*

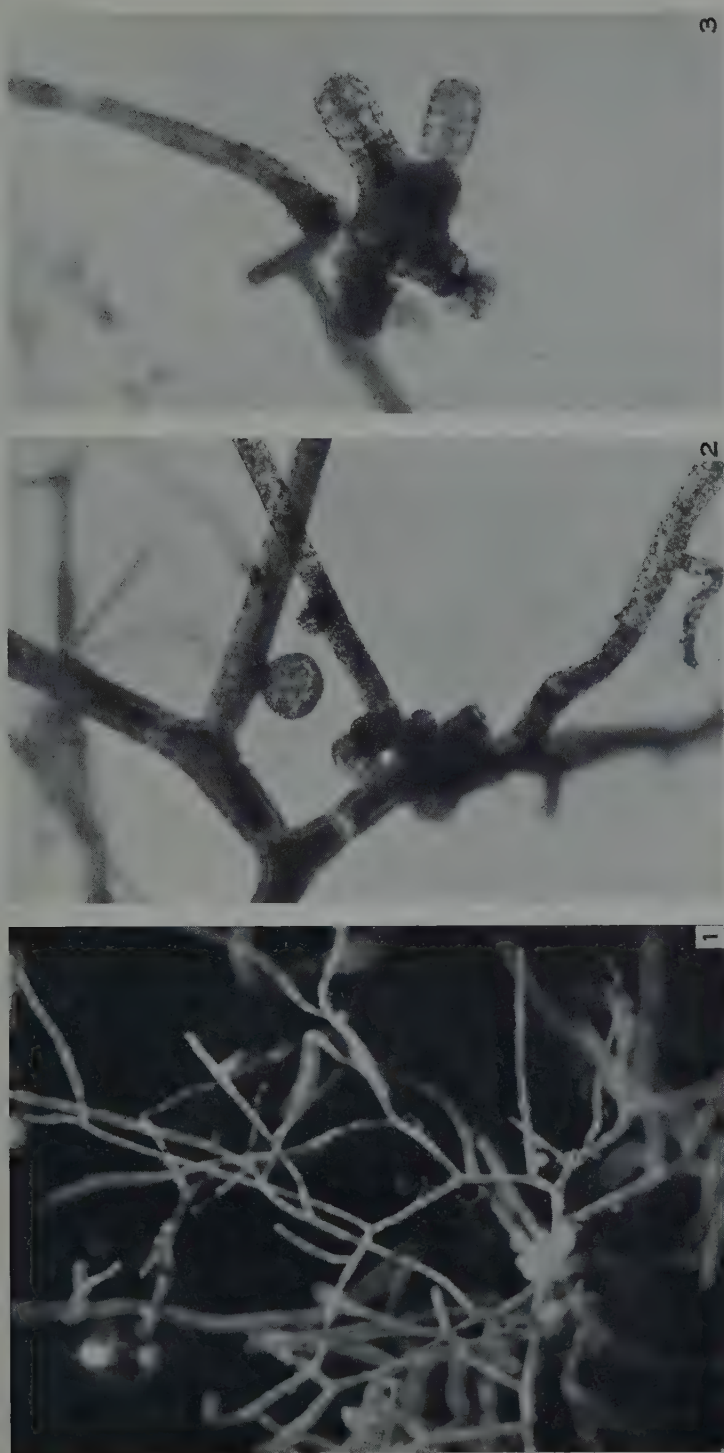


Fig. 1.—Filamentous prothallus of *Polyphlebium venosum* with antheridia and archegonia on an archegoniophore. $\times c. 30$.

Fig. 2.—Antheridia. $\times 165$.

Fig. 3. Archegoniophore with archegonia. $\times 165$.

- HOLLOWAY, J. E. (1944).—The gametophyte, embryo, and developing sporophyte of *Cardiomanes reniforme* (Forst.) Presl. *Trans. Roy. Soc. N.Z.* **74**: 196–206.
- METTENIUS, G. (1864).—Ueber die Hymenophyllaceen. *Abh. Sächs. Ges. Wiss.* **7**: 401–501.
- STOKEY, ALMA G. (1940).—Spore germination and vegetative stages of the gametophytes of *Hymenophyllum* and *Trichomanes*. *Bot. Gaz., Lond.* **101**: 759–90.
- STOKEY, ALMA G. (1948).—Reproductive structures of the gametophytes of *Hymenophyllum* and *Trichomanes*. *Bot. Gaz., Lond.* **109**: 363–80.

APPENDIX I

KEY TO THE VICTORIAN SPECIES OF THE FAMILY HYMENOPHYLLACEAE

- A. Indusium tubular, receptacle exserted (*Trichomanes* group)B
 Indusium valvate, receptacle usually included (*Hymenophyllum* group)C
- B. Rhizome filiform, fronds remote, once pinnate, veins branched within lobed segments
Polyphlebium venosum
 Rhizome stouter, fronds clustered, bipinnate, rachis winged, segments very narrow, each with a single vein*Macroglena caudata*
- C. Margin of lamina toothedD
 Margin of lamina entireE
- D. Lips of indusium toothed*Hymenophyllum cupressiforme*
 Lips of indusium entire, pinnae pinnatifid on upper side only*Hymenophyllum peltatum*
- E. Fronds minute, simple, black-edged*Craspedophyllum marginatum**
 Fronds pinnately dividedF
- F. Indusium subtended by conspicuous branches of the vein. Rachis but not stipe winged. Pressed specimens have distinct odour*Mecodium rarum*
 Indusium not subtended by conspicuous branches of the veinG
- G. Rachis and stipe conspicuously winged*Mecodium australe*
 Rachis terete in lower part, pinnae flabellately divided*Mecodium flabellatum*

*Not in Victoria, but in Tasmania, New South Wales, and Queensland.

HETEROPOLOID TWINS AND APOMIXIS IN *CASUARINA NANA* SIEB.

By B. A. BARLOW*

[Manuscript received March 14, 1958]

Summary

Casuarina nana Sieb. occurs in some localities as a diploid, with a somatic chromosome number of 22, and in others as a tetraploid ($2n = 44$), and there is evidence of hybridization between these two chromosome forms. In one population of this species diploid, triploid, and tetraploid plants occur together. Heteroploid twins, with 33 and 44 chromosomes respectively, are regularly produced in seeds from the triploids. A reproductive system involving apomixis is suggested, and supported by embryological evidence.

A similar situation apparently exists at other localities. These populations may have resulted from hybridization between the diploid and tetraploid forms, and illustrate a stage in the development of obligatory apomixis.

I. INTRODUCTION

Casuarina nana is a low or medium-sized shrub which has an irregular distribution in central and southern New South Wales. It occurs in isolated, often widely separated populations on exposed ridges in the coastal ranges and the eastern parts of the Great Dividing Range, and rises to altitudes of over 3000 ft. The species appears to be almost perfectly dioecious.

Preliminary investigations were carried out by the writer on batches of seed from a few localities, with the original aim of determining the chromosome number of the species. It was found that the chromosome number varied both within and between populations. Seedlings from near Lithgow had somatic numbers ranging from 20 to 24, whilst at National Park, numbers of *c.* 33 and *c.* 44 were recorded.

It was also noted that in some populations the occurrence of twin seedlings within one seed was quite frequent, and the two members of each twin pair were found to differ in chromosome number. Because of the variation in chromosome number in the species, and the apparent occurrence of heteroploid twins, a more intensive cytological study of *Casuarina nana* has been made.

II. MATERIALS AND METHODS

Individual plants were labelled or sampled at a number of sites. Seeds were collected from the female plants to determine the chromosome numbers of the progeny, and the pollen fertility of the male plants was estimated from semi-permanent pollen preparations, acid fuchsin being used as a cytoplasmic stain. In some cases the somatic chromosome numbers of the labelled plants were also determined.

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The chromosome numbers of seedling progenies were found from aceto-orcein squash preparations of root tips, after pretreatment with *p*-dichlorobenzene. The chromosome numbers of mature plants were determined by making similar squashes of meristematic tissue dissected out from the apices of actively growing axillary shoots, which had also been pretreated with *p*-dichlorobenzene.

For embryological study, individual carpels were dissected from young female cones, embedded in paraffin, and sectioned at 10 μ .

TABLE 1
CHROMOSOME NUMBERS OF PROGENIES OF FEMALE PLANTS OF CASUARINA
NANA, LITHGOW-NEWNES

| Plant No. | Chromosome Numbers of Progeny | | | | | | | |
|-----------|-------------------------------|----|-----|----|----|----|----|----|
| | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 33 |
| L11 | | 1 | 7 | 1 | 1 | | | |
| L12 | 2 | 1 | 8 | 1 | | | | |
| L13 | | | 10 | 1 | | | | 1 |
| L14 | | | 7 | 3 | | | | |
| L15 | | | 8 | | 1 | | | 1 |
| L16 | 1 | 1 | 9 | | | | | |
| L17 | | | 11 | | | | | 1 |
| L18 | | | 10 | | | | | |
| L19 | | | 8 | 1 | | | | 1 |
| L20 | | | 10 | | | | | |
| N1 | | | 10 | 2 | | | | |
| N2 | | | 10 | | | | | |
| N3 | | | 3 | | | | | |
| N4 | | | 10 | | | | | |
| N5 | | | 10 | | | | 1 | |
| N6 | | | 7 | 1 | | | | |
| N7 | | | 9 | 1 | | | | |
| N8 | | | 9 | 1 | | | | |
| N9 | | 1 | 11 | | | | | |
| N10 | | | 12 | | | | | |
| Total | 3 | 4 | 179 | 12 | 2 | 0 | 1 | 4 |

III. OBSERVATIONS AND DISCUSSION

(a) Chromosome Numbers

(i) *Lithgow-Newnes*.—Ten male and 10 female plants were labelled at each of two sites between Lithgow and Newnes. The chromosome numbers of approximately 10 seedlings from each female plant were counted. The results are listed in Table 1.

It is evident that the normal chromosome number for this population is 22, as most of the progeny of each labelled plant had this number. However, the aneuploids and triploids occur with a significantly high frequency and indicate that con-

ditions such as incomplete pairing, causing unequal segregation of chromosomes, and non-reduction are present at meiosis.

Male and female plants occur in approximately equal numbers. Most of the male plants examined had a high frequency of apparently functional pollen, but two were completely sterile. Somatic chromosome counts were made on two male plants, Nos. L2 (pollen fertility 97 per cent.) and L3 (1 per cent.), and both were found to have $2n=22$.

No twin seedlings were recorded in this population. It appears to be a normal diploid one, the chromosome number being $2n = 22$, but with occasional sterility and some variation in chromosome number, showing slight cytological unbalance. It seems probable that aneuploid seedlings do not normally survive in the field.

TABLE 2
CHROMOSOME NUMBERS OF PROGENIES OF FEMALE PLANTS
OF CASUARINA NANA, PIGEON HOUSE RANGE

| Site | Plant No. | Chromosome Numbers of Progeny | |
|-------|-----------|-------------------------------|----|
| | | 33 | 44 |
| 1 | 1 | 3 | 10 |
| | 2 | — | 10 |
| | 3 | — | 12 |
| | 4 | — | 13 |
| 2 | 5* | — | 11 |
| | 6 | 2 | 9 |
| 3 | 7 | 4 | 2 |
| Total | | 9 | 67 |

*One heteroploid (22-44) twin pair was also recorded in the progeny of this plant.

(ii) *Pigeon House Range*.—Seed samples were taken from seven plants at three sites in Pigeon House Range, south-west of Nowra. The chromosome numbers of the seedling progenies are listed in Table 2.

The chromosome numbers of the progenies indicate that this is a tetraploid population, with a somatic chromosome number of 44. Triploid seedlings were recorded among the tetraploids in three samples, representing each of the three sites. They suggest that diploids must be present in this or an adjacent population, even though they have not been recorded, and that hybridization is taking place.

Male and female plants occur with approximately equal frequency. Plants of each kind occur in clumps up to 10 yards in diameter, which suggests that the tetraploid form is able to vegetatively propagate itself by budding from the roots. This condition is known in *C. cunninghamiana*, *C. glauca*, and a number of other species (L. A. S. Johnson, personal communication).

In nearly all cases, seed from the locality contained only single functional embryos. However, one twin pair of seedlings was produced in the progeny of plant No. 5. It was heteroploid, one seedling being a diploid ($2n = 22$) and the other a tetraploid ($2n = 44$). This occurrence is of some significance in relation to the occurrence of heteroploid twins in another population, and is discussed later.

TABLE 3
CHROMOSOME NUMBERS OF SELECTED PLANTS OF CASUARINA NANA, WENTWORTH
FALLS-KING'S TABLELAND
Determined from shoot apices

| Female Plants | | Male Plants | | |
|---------------|-------------------|-------------|-------------------|----------------------|
| Plant No. | Chromosome Number | Plant No. | Chromosome Number | Pollen Fertility (%) |
| KT1 | 33 | KT20 | 22 | > 95 |
| KT2 | 33 | KT21 | 33 | 0 |
| KT3 | 44 | KT22 | 22 | 80 |
| KT4 | 44 | KT23 | 22 | > 95 |
| KT5 | 33 | KT24 | 33 | 0 |
| KT6 | 33 | KT25 | 22 | 95 |
| KT7 | 33 | KT26 | 22 | 90 |
| KT8 | 33 | KT27 | 22 | 50 |
| KT9 | 33 | KT28 | 22 | 90 |
| KT10 | 33 | KT29 | 22 | 75 |
| KT11 | 22 | KT30 | 22 | > 95 |
| KT12 | 22 | KT31 | 22 | 80 |
| KT13 | 22 | KT32 | 33 | < 35 |
| KT14 | 33 | KT33 | 22 | > 95 |
| KT15 | 33 | KT34 | 22 | > 95 |
| KT16 | 33 | KT35 | 33 | 0 |
| WF11 | 33 | KT36 | 33 | 0 |
| WF12 | 33 | KT37 | 33 | 0 |
| WF13 | 33 | KT38 | 22 | 70 |
| WF14 | 33 | KT39 | 22 | > 95 |
| WF15 | 33 | WF1 | 22 | 60 |
| WF16 | 33 | WF2 | 33 | < 25 |
| WF17 | 33 | (KT4)* | (44) | (95) |
| WF18 | 33 | | | |
| WF19 | 33 | | | |
| WF20 | 33 | | | |
| WF21 | 33 | | | |

*Male branch on KT4, i.e. a branch showing sex reversal.

(iii) *Wentworth Falls-King's Tableland*.—There is an extensive population of *C. nana* in the Wentworth Falls-King's Tableland area, spreading along the high ridges. About 50 plants in this area were labelled, and their chromosome numbers determined. The numbers are listed in Table 3.

The male plants examined were all found only after an extensive search, and they are actually greatly outnumbered by female plants, and probably constitute less than 5 per cent. of the population. Of the female plants, triploids are the most frequent. Tetraploids are apparently rare and occur with the triploids. The diploids were all found in one small group, and their frequency in the population as a whole could only be estimated from a much more extensive survey.

TABLE 4
CHROMOSOME NUMBERS OF PROGENIES OF TRIPLOID FEMALE PLANTS OF
CASUARINA NANA, WENTWORTH FALLS-KING'S TABLELAND

| Plant No. | Chromosome Numbers of Progeny | | |
|-----------|-------------------------------|-------------|------------------|
| | Single 33's | Single 44's | 33-44 Twin Pairs |
| KT1 | 3 | 3 | 3 |
| KT2 | 5 | 1 | 2 |
| KT5 | 2 | 2 | 3 |
| KT6 | 1 | 3 | 3 |
| KT7 | 7 | 1 | 1 |
| KT8 | 2 | 2 | 3 |
| KT9 | 4 | 2 | 2 |
| KT10 | 1 | 1 | 4 |
| WF11 | 3 | 3 | 3 |
| WF12 | 6 | — | 2 |
| WF13 | 7 | 1 | 2 |
| WF14 | 5 | — | — |
| WF15 | 10 | 1 | — |
| WF16 | 4 | 2 | 1 |
| WF17 | 6 | 2 | 1 |
| WF18 | 6 | — | 2 |
| WF19 | 5 | — | 1 |
| WF20* | 6 | 3 | 1 |
| Total | 83 | 27 | 34 |

*One pentaploid seedling was also recorded.

Further examinations of each chromosome form are described below.

(1) *The Diploids*.—Most of the male plants are diploids, and they have a high percentage of apparently functional pollen (see Table 3). From the progenies of the diploid females the chromosome numbers of 14 seedlings were determined, and all were diploid ($2n = 22$). There is probably little or no fertilization by other than haploid pollen, primarily because almost all of the functional pollen is produced by the diploid plants. This means that the diploid stock is able to maintain itself in the population, despite the presence of triploid and tetraploid plants.

(2) *The Triploids*.—A number of the male plants are triploids, and these have high or complete pollen sterility (Table 3). The progenies of 18 female plants were examined, and all were found to be of the same type. In each case both triploid and tetraploid seedlings were produced (see Table 4). Many twin pairs were produced and altogether constituted 27 per cent. of germinating seeds. This is a far greater frequency than in any of the genera studied by Muntzing (1937, 1938), where the frequency of twins is usually less than 1 per cent. *In every case it was found that one member of each twin pair was a triploid, whilst the other was a tetraploid.* If the formation of triploids and tetraploids takes place in a random fashion, a high proportion of both-triploid and both-tetraploid twin pairs would be expected. Since this is not the case it appears that there is a regular reproductive system producing the heteroploid twins, and it is suggested that the single seedlings (which are either triploid or tetraploid) represent cases where one of the twin embryos has failed to develop, owing to competition. The regular (100 per cent.) occurrence of heteroploids

TABLE 5
CHROMOSOME NUMBERS OF PROGENIES OF TETRAPLOID
FEMALE PLANTS OF CASUARINA NANA, WENTWORTH
FALLS-KING'S TABLELAND

| Plant No. | Chromosome Numbers Of Progeny | |
|-----------|----------------------------------|----|
| | 33 | 44 |
| KT3 | 8 | — |
| KT4* | 5 | 5 |

*One heteroploid (22-44) twin pair was also recorded in the progeny of this plant.

in the twin pairs of triploid *C. nana* is in contrast to the observations of Muntzing (1937, 1938), where heteroploid twin pairs averaged only 5 per cent. of the observed twin sets.

(3) *The Tetraploids*.—No tetraploid male plants have been recorded. However, they may be present, as they would be essential for normal sexual reproduction in the tetraploids. With the reproductive system which is suggested below for this population, the occurrence of a pentaploid seedling ($2n = 55$; see Table 4) in the progeny of triploid plant No. WF20 also indicates that diploid pollen is produced in small amounts.

The only tetraploid male material observed was a male branch on one of the two tetraploid female plants found, No. KT4. The species has been otherwise found to be perfectly dioecious, and this occurrence on a tetraploid plant suggests that sex determination at the tetraploid level may be less precise than in diploids, so that partial or complete sex reversal may be more likely to occur. The pollen fertility of this material was 95 per cent.

The chromosome numbers of the progenies of the two tetraploid female plants found are listed in Table 5. They show that these plants are probably similar in

reproductive behaviour to those on Pigeon House Range. The much higher frequency of triploids in the progenies as compared with those of Pigeon House Range is consistent with the high rate of hybridization which would be expected between the rare tetraploids and the more common diploids.

One heteroploid twin pair was recorded. It was similar to that from Pigeon House Range, the chromosome numbers of the two seedlings being 22 and 44.

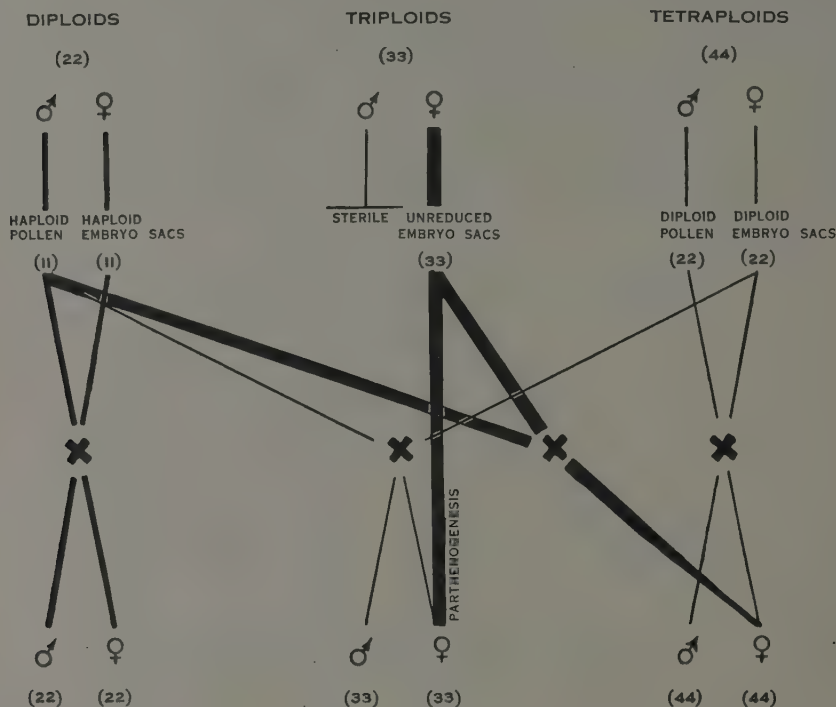


Fig. 1.—Diagram of the breeding system of *Casuarina nana*, Wentworth Falls-King's Tableland. The thicknesses of the lines indicate the frequency of each type in population, and the frequency of each type of reproduction. Chromosome numbers are shown in brackets.

(b) Apomixis

Within the Wentworth Falls-King's Tableland population, then, there are some normal diploids with 22 chromosomes, the males having high pollen fertility and the females producing normal diploid progeny. The majority of the plants, however, are triploids, there being a few sterile males, and many females, which produce in their progeny both triploids and tetraploids, and heteroploid twins. There are rare tetraploid plants in the population. The data strongly suggest that apomixis is involved in the reproductive system of the triploids and the following hypothesis is tendered, and has been further examined in relation to embryo sac and embryo development.

If non-reduction takes place in the embryo sac mother cell (e.s.m.c.) meiosis of the triploid females, then triploid embryo sacs would be produced in each ovule. (In *Casuarina* each ovule usually contains many embryo sacs.) Parthenogenetic development would then result in a triploid embryo, whilst fertilization by a haploid male nucleus would produce a tetraploid embryo. The regular occurrence of

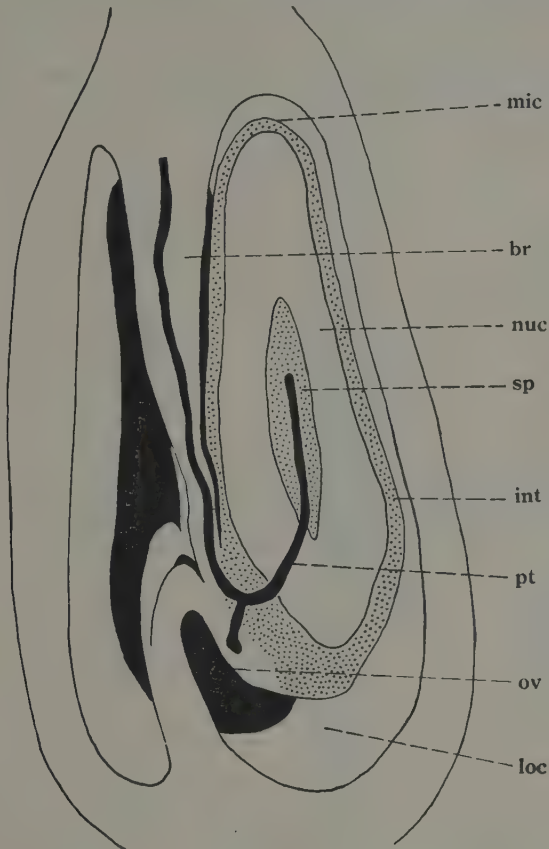


Fig. 2.—Diagram of the ovary and ovules of *Casuarina nana*, showing the course of the pollen tube. *mic*, Position of micropyle; *br*, bridge; *nuc*, nucellus; *sp*, sporogenous tissue; *int*, integument; *pt*, course of pollen tube; *ov*, the other ovule, partly obscured (in black); *loc*, loculus.

heteroploidy in the twin pairs could be explained by assuming that parthenogenetic development of one cell only takes place when stimulated by fertilization of another. Single seedlings would result from the suppression of one twin by the other.

This scheme, involving apomixis and sexual reproduction together, is summarized in Figure 1. The suggested apomixis depends on the ability of the female plants to produce unreduced embryo sacs, and to initiate parthenogenetic development.

(c) *Embryogenesis*

The embryogenesis of the triploid plants has been examined as a means of testing the above hypothesis. The observations were made on serial sections cut from carpels which were dissected from young cones.

The structure of the ovary in *C. nana* is the same as that of species previously described (Treub 1891; Frye 1903; Juel 1903; Swamy 1948). There are two epitropous ovules of equal size and each is connected to the base of the style by a bridge of tissue arising from the chalaza near the point of attachment of the funiculus (Fig. 2). There is a copious sporogenous tissue forming a central mass in each ovule.

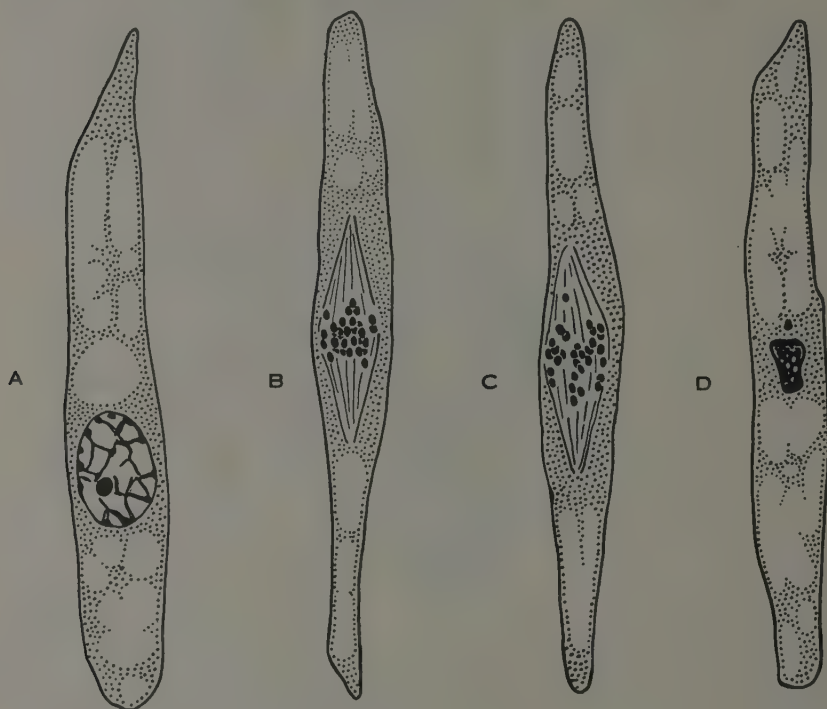


Fig. 3.—Restitutive e.s.m.c. meiosis in triploid *Casuarina nana*. A, prophase. Most mother cells were at this stage. B, C, metaphase I, showing failure of organization of a plate. The two figures illustrate the extremes of chromosome distribution in the spindle. D, a probable restitution nucleus, in this case with some excluded chromatin. All $\times c. 600$.

The e.s.m.c. meioses in the triploids appear to be restitutive. The majority of the nuclei in the mother cells were in the meiotic prophase condition (Fig. 3A), in which they apparently remain for a considerable length of time, but a number of cells were observed at metaphase I. At this stage the chromosomes are aggregated in the middle of the spindle mostly as univalents, and are not organized into a plate (Fig. 3B, C). In over 170 such configurations observed, there was some variation in the compactness of the groups of chromosomes, but there was never a regular organization on the equatorial plane. Only two anaphase I separations were observed

and, compared with the number of metaphase configurations seen, this indicates that a reduction division is almost non-existent. A number of cells contained nuclei which are interpreted as restitution nuclei; they were unlike the prophase nuclei, and appeared to be metaphase groups reverting to the resting stage (Fig. 3D). These nuclei did not have the dumb-bell shape common among restitution nuclei, but this would not be expected if there is normally no anaphase separation.

It is quite likely, therefore, that the triploids produced unreduced embryo sacs. Unfortunately no stages in the development of the embryo sacs were observed, and the earliest post-meiotic stages examined are at about the time of fertilization. In these, few megaspores can be seen to have developed past the uninucleate stage, and usually only one embryo sac develops to maturity. In other species previously studied in this respect, up to 20 mature embryo sacs have been reported in one ovule (Treub 1891; Frye 1903; Swamy 1948).

When pollination takes place the ovary is solid and undifferentiated, having neither loculus nor ovules. Fertilization takes place at least 2 months later, and in ovules at this stage the pollen tube can be traced back from the embryo sac through the chalaza, and a short distance up the bridge (Fig. 2). The rest of its length is not apparent, and is probably destroyed by growth and development in the ovule subsequent to pollination.

The pollen tube probably grows down the bridge and into the chalaza, where it stays until the embryo sac is mature. A few short, blind branches are often produced in this region. When an embryo sac matures, the pollen tube grows directly to it, sometimes producing a simple branch or loop in the process (Figs. 4A, B). The pollen tube grows to the egg apparatus either inside or outside the embryo sac to be fertilized. This behaviour occurs in *C. stricta* (Frye 1903), but not in *C. equisetifolia* or *C. montana* (Swamy 1948).

Occasionally no embryo sacs in an ovule reach maturity. When this happens the pollen tube produces a large number of loops and branches in the region where the fertile embryo sac would normally be (Fig. 4C).

The pollen tube is thick-walled and of large diameter, being at least as wide as the functional embryo sac. It crushes most of the surrounding cells when it approaches the embryo sac, particularly when it produces a branch. The embryo sac has a normal egg apparatus of two synergids and an ovum, and two polar nuclei. From the preparations available, it is not possible to state whether or not antipodals are present.

Embryo development commences when there are a few free endosperm nuclei present. Twin embryos develop close together within the one endosperm mass, and obviously arise from a single embryo sac. They probably develop from a fertilized ovum (with 44 chromosomes) and parthenogenetically from a synergid (33 chromosomes). This phenomenon is known in a number of angiosperm species (Cooper 1943; Maheshwari 1950). The synergid possibly divides before the zygote (see Fig. 4B), so that the larger embryos may be the triploid ones. Very unequal pairs were seen a few times and single embryos were common, so that one embryo may often suppress the other at a very early stage.

The endosperm enlarges quickly, and is free nucleate at first. Wall formation commences at the micropylar end, and eventually the whole endosperm becomes cellular. In one ovule a two-chambered endosperm was produced, possibly through a cleavage of the embryo sac after the first endosperm mitosis (Fig. 5A). The embryo or embryos develop in the normal dicotyledon manner (Fig. 5).

The population in the Wentworth Falls-King's Tableland area was unfortunately destroyed by fire during the time this study was being made, and the observations of the post-meiotic stages, particularly those near fertilization, come from a very limited number of preparations. However, from the available material the essential

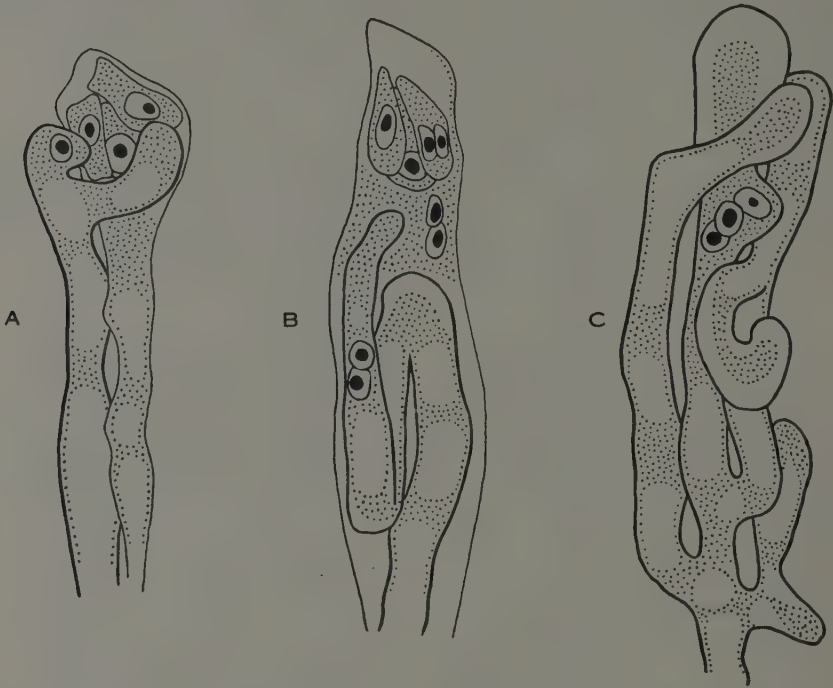


Fig. 4.—Pollen tube of *Casuarina nana*: A, outside the functional embryo sac, showing a branch near the tip; B, inside the functional embryo sac and containing a loop; C, in an ovule without a functional embryo sac, showing many branches and loops. In B, a synergid has apparently divided before the zygote. \times c. 300.

observations have been made. The pollen tube reaches the embryo sac and apparently fertilizes it, and twin embryos subsequently develop within one embryo sac, probably one from the zygote and the other from a synergid.

(d) History of the Wentworth Falls-King's Tableland Population

The course of development of the present population of *C. nana* at Wentworth Falls-King's Tableland is not clear. The entire population may have arisen at this locality from the diploids, the first triploids having been produced through fertilization involving unreduced gametes. As this would probably be a recurrent process, triploids with apomictic ability could have arisen from time to time, and finally

become established in the population. The tetraploids could have been produced at any stage after the triploids appeared, so that all three polyploid levels would be represented. Selection would result in changes in the frequency of each form, and if this process took place in different areas, different stabilized populations could be produced, through selection for a particular form.

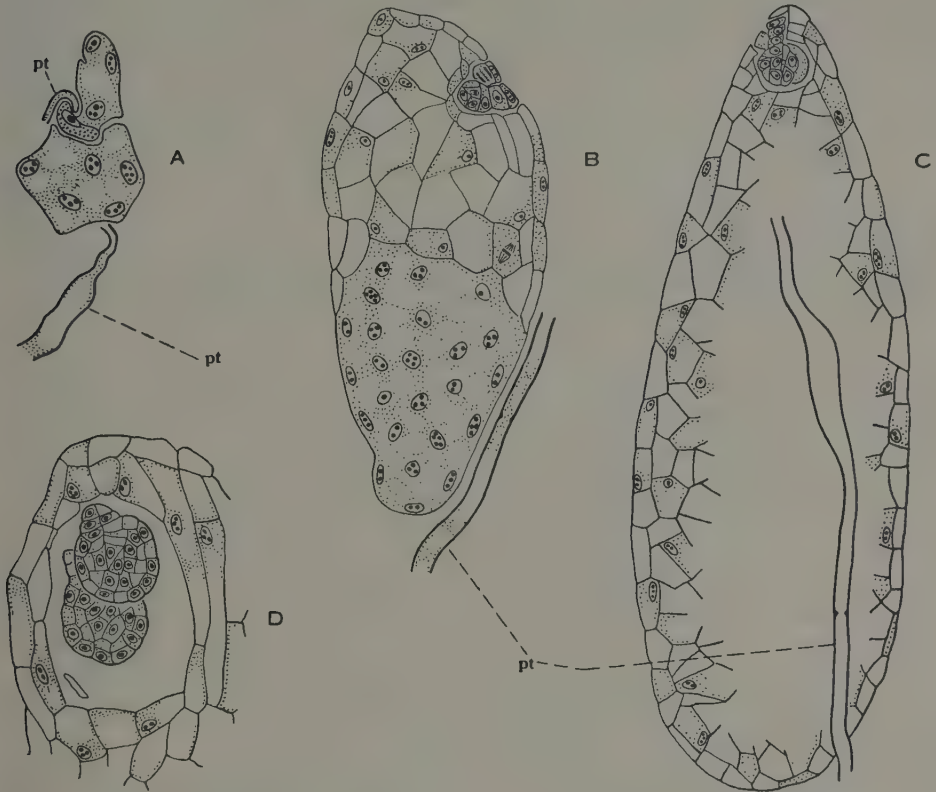


Fig. 5.—Endosperm and embryo development. *A*, two-chambered endosperm of a few free nuclei; *pt*, pollen tube. *B*, micropylar end of endosperm cellular; chalazal end free nucleate. Note twin embryos developing side by side. *C*, endosperm completely cellular. Note pollen tube within the endosperm, indicating fertilization from within the embryo sac. *D*, twin embryos, later stage. $\times c. 50$.

Alternatively, this locality may be a place where hybridization has taken place between a diploid and a tetraploid form, thus producing the triploids. Diploid and tetraploid races are known in other New South Wales species of *Casuarina*, and hybridization often occurs where the races come in contact (Barlow 1958, and unpublished data).

The suggested reproductive behaviour of the triploids, which has been supported by evidence on embryogenesis, shows that there is an essential connexion between the triploids and the other forms. Fertile male plants are necessary for the reproduction of the triploids, but are not produced in the progeny of the triploids, and

hence must be replaced in the population from an outside source. The triploids depend mainly on the survival of the diploid stock for the production of fertile male plants, but may be similarly linked with the tetraploids. The occurrence of a pentaploid seedling shows that diploid pollen can fertilize the embryo sacs of the triploid, and the rarity of pentaploid seedlings is probably due to the rarity of tetraploid male plants. This connexion between the triploids and the other forms suggests that the diploids and possibly the tetraploids were established in the population before the triploids. Furthermore, the results show that at the present time the tetraploids are hybridizing with the diploids, so that even if the original triploids were not produced through hybridization, many which have been produced subsequently have had a hybrid origin.

The present breeding system of the triploids is simply the result of non-reduction at megasporogenesis, coupled with the capacity for parthenogenetic development of a synergid after fertilization. This system has naturally resulted in the unbalance between male and female plants. The apomictically produced triploids would have the same genetic constitution as their parent plants, and hence normally be female, although occasional sex reversal may occur. Triploid male plants could otherwise be produced as hybrids between diploids and tetraploids, but even then their formation depends on a suitable balance between male-determining and female-determining chromosomes or chromosome segments.

The ability for parthenogenetic development of a synergid is apparently also present in the tetraploid stock. As described above, two heteroploid twin pairs have been recorded in the progenies of tetraploids—one from Pigeon House Range and the other from King's Tableland. One seedling of each was a tetraploid, apparently being derived from a normal zygote, and the other a diploid, as would be expected if it was derived from an unfertilized synergid. The frequency of occurrence of these twin pairs in the tetraploid (two out of 300 germinated seeds) is far less than in the triploids, as would be expected, since the sexual sterility of the triploids makes the ability for synergid parthenogenesis essential for their survival.

The present abundance of triploids in the population indicates that they may be more vigorous than the diploids and tetraploids, and may be expanding at their expense. This is supported by the fact that the mean frequency of tetraploid plants in the seedling progenies (38 per cent.) is considerably greater than the frequency of tetraploids in the mature plants (less than 10 per cent.), showing that within the progeny of the triploids the triploids are the more vigorous.

A number of twin pairs were grown without separation until one member of each pair suppressed the other. The chromosome number of the survivor was counted. In the few observations made all the survivors were triploids, which further supports the suggestion that the triploids have the competitive advantage. In this case then, it is the form at the lower polyploid level which is the more vigorous, in contrast with *C. littoralis* (Barlow 1958) and many other species of angiosperms (Stebbins 1947), where "aggressiveness" is associated with higher polyploid levels.

This is another example of a species in which an aggressive form is maintained by apomixis. If, through some mutation (in the wider sense), the necessity for fertilization to stimulate parthenogenesis is dispensed with, then, at a single step,

the triploids will become obligatory apomicts. The necessity for diploid male plants will disappear, and populations consisting solely of triploid female plants could exist.

An alternative hypothesis is that the triploids are normally apomictic, producing only triploid progeny, and that the presence of diploid stock has caused a breakdown in this system. This population can therefore be explained either as a stage in the development of apomixis or as an instance of its breakdown. The former seems more likely, as the latter would mean a reversal from a rigid apomictic system to a more flexible one of outbreeding.

This process would of course not be limited to dioecious angiosperms. It is a sequence which many plant forms may have followed in becoming obligatory apomicts.

TABLE 6
CHROMOSOME NUMBERS OF PROGENIES OF PLANTS OF CASUARINA NANA,
FROM NATIONAL PARK AND MOUNT BANKS

| Locality | Chromosome Numbers of Progeny | | |
|----------------|-------------------------------|-------------|------------------|
| | Single 33's | Single 44's | 33-44 Twin Pairs |
| National Park: | | | |
| Sample 1 | 8 | 2 | — |
| Sample 2 | 5 | 5 | 3 |
| Mount Banks | 3 | 1 | 2 |

(e) *Other Localities*

Two other populations have been found in which the seedling progenies are similar to those at Wentworth Falls-King's Tableland, consisting of triploid and tetraploid seedlings, and heteroploid twins. They occur at National Park and Mount Banks (see Table 6). On the assumption that the reproductive systems in these populations are the same as or similar to that described, it is clear that the triploid-tetraploid apomictic form is not restricted to a small area or a single population, and has probably arisen independently a number of times.

(f) *The Significance of Apomixis in Casuarina nana*

Records of apomixis in dioecious plants are rare. Gentry (1955) has suggested that *Simmondsia chinensis* and *Piper nigrum* can reproduce apomictically, but the data are limited to observations that the species sometimes set seeds in the apparent absence of male plants. *Casuarina nana* is perhaps unusual, then, in that it is a dioecious species which is apparently apomictic. Nevertheless the species is similar to a great number of other apomicts, in which the occurrence of apomixis is associated

with the higher polyploid levels within a genus or within the one species, and provides a short-term escape from sterility and extinction (Darlington 1939; Stebbins 1950).

In Australian plants apomixis has only been described or suggested a few times. In view of the many unusual cytological and taxonomic conditions which are being found in native plants, it is probable that apomixis has played a wider part in the development of the present Australian flora than the current information suggests. Only when further investigations have been made on the breeding systems of Australian plants will the importance of apomixis be known.

IV. CONCLUSIONS

(1) *Casuarina nana* occurs at Lithgow as a sexually reproducing, dioecious species with a somatic chromosome number of 22, and at Pigeon House Range as a tetraploid ($2n = 44$). The two forms apparently hybridize.

(2) At Wentworth Falls and King's Tableland, diploids form only a small proportion of the population. Most plants are triploid females, which produce triploids and tetraploids and triploid-tetraploid twins in their progenies. Triploid male plants, which are sterile, and tetraploid plants also occur.

(3) It is suggested that the triploids are facultative apomicts, and produce unreduced (triploid) embryo sacs. Fertilization by haploid pollen produces tetraploid embryos whilst triploid embryos are the result of pseudogamy and parthenogenetic development. Heteroploid twins result from the development of one embryo of each kind in an ovule.

(4) This theory is supported by the observations of probable restitution in the e.s.m.c. meioses, and of twin embryos developing in an apparently fertilized embryo sac, one from a fertilized egg and the other probably from an unfertilized synergid.

(5) It is clear that this type of reproduction in triploids can only take place in the presence of fertile male plants, i.e. in the presence of diploid or tetraploid stock. The population may have developed originally from a hybridization between diploid and tetraploid forms of *C. nana*.

(6) Evidence from frequencies of mature plants, the growing of twin seedlings, and embryogenesis suggests that the triploid seedlings are more vigorous than the tetraploids.

(7) Such populations occur in other areas, and have probably arisen independently a number of times.

V. ACKNOWLEDGMENTS

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VI. REFERENCES

- BARLOW, B. A. (1958).—Diploid and tetraploid *Casuarina littoralis* Salisb. (syn. *C. suberosa* Ott. & Dietr.). *Aust. J. Bot.* **6**: 38–46.
- COOPER, D. C. (1943).—Haploid-diploid twin embryos in *Lilium* and *Nicotiana*. *Amer. J. Bot.* **30**: 408–13.

- DARLINGTON, C. D. (1939).—"The Evolution of Genetic Systems." (Cambridge Univ. Press.)
- FRYE, T. C. (1903).—The embryo sac of *Casuarina stricta*. *Bot. Gaz.* **36**: 101-13.
- GENTRY, H. S. (1955).—Apomixis in black pepper and jojoba? *J. Hered.* **46**: 8.
- JUEL, H. O. (1903).—Ein Beitrag zur Entwicklungsgeschichte der Samenlagen von *Casuarina*. *Flora, Jena* **92**: 284-93.
- MAHESHWARI, P. (1950).—"An Introduction to the Embryology of Angiosperms." (McGraw-Hill: New York.)
- MUNTZING, A. (1937).—Polyploidy from twin seedlings. *Cytologia, Tokyo, Fujii Jub. Vol.*, pp. 211-27.
- MUNTZING, A. (1938).—Note on heteroploid twin plants from eleven genera. *Hereditas, Lund* **24**: 487-91.
- STEBBINS, G. L. (1947).—Types of polyploids: their classification and significance. *Advanc. Genet.* **1**: 403-29.
- STEBBINS, G. L. (1950).—"Variation and Evolution in Plants." (Columbia Univ. Press: New York.)
- SWAMY, B. G. L. (1948).—A contribution to the life history of *Casuarina*. *Proc. Amer. Acad. Arts Sci.* **77**: 1-32.
- TREUB, M. (1891).—Sur les Casuarinées et leur place dans le système naturel. *Ann. Jard. Bot. Buitenzorg* **10**: 177-200.

CYCLONES AS AN ECOLOGICAL FACTOR IN TROPICAL LOWLAND RAIN-FOREST, NORTH QUEENSLAND

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[Manuscript received April 8, 1958]

Summary

The influence of cyclones on the structural and floristic composition of tropical lowland and foothill rain-forest in north Queensland is briefly described. Local topographic effects, as well as the general frequency and intensity of cyclones, are important. The local intensification of wind velocities, probably exceeding 100 m.p.h., which occur regularly in parts of the coastal corridor south of Cairns, produces "cyclone scrubs". Because of extensive windthrows, these have a low uneven canopy with scattered emergents densely draped by vines.

In more sheltered areas, with cyclonic winds averaging 60–80 m.p.h. not locally accelerated by turbulence, upper canopy defoliation and occasional windthrows have resulted in a dense understorey of the shade-intolerant lawyer vine (*Calamus australis* (Mart.) Beccari) under a relatively even canopy averaging 90–110 ft high.

On exposed spurs of the rugged coastal ranges, *Acacia aulacocarpa* A. Cunn. is a common emergent of vine forests. Fire following "dry" cyclone damage may further modify vine forests adjacent to fire-paths in sclerophyll or grassy forest. The principal effects of fire are the absence of the fire-sensitive *Calamus* spp., and the presence of numerous sclerophyllous species in addition to *A. aulacocarpa*.

The reactions of Queensland tropical rain-forest to cyclones are compared with those described for west Africa, Malaya, and the West Indies.

The catastrophic effect of cyclones on rain-forests overrides the usual ecological factors, and in such areas, even without human interference, a stable forest climax is not attained.

The frequency of cyclonic damage means that exposed sites of the tropical lowlands and adjacent foothills of north Queensland have an uncertain silvicultural future.

I. INTRODUCTION

Tropical cyclones with wind speeds in excess of 35 m.p.h. (40 knots) are regularly experienced along the north-east coast of Australia. Such cyclones usually originate in the Coral Sea region between about 8 and 18° S. (Gabites 1956) and are most frequent during the months of January, February, and March (Brunt and Hogan 1956). During the period 1867–1939, "particularly disastrous storms" in tropical Queensland were recorded in 1884, 1896, 1899, 1903, 1911, 1918, 1920, 1923, 1928, and 1934 (Anon. 1940). The most destructive cyclones in recent times occurred on March 10, 1918, and March 6, 1956, in the tropical rain-forests of the Innisfail region, north Queensland.

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Observations since 1945 on certain north Queensland lowland and foothill forests little disturbed by man had suggested that "scrubs" characterized by low uneven canopies and dense vine tangles, and taller rain-forests with dense vine understories or with scattered sclerophyll emergents, were related in some way to past cyclone damage. The severe cyclone of March 1956 provided an opportunity to study at first hand the nature of cyclone damage to vegetation and to interpret the resulting changes in terms of succession and of the silvicultural potentialities in the area.

A conspicuous physiographic feature of north Queensland is the discontinuous coastal range of mountains, averaging 2000–3000 ft high, which reaches its highest point at Bartle Frere (5275 ft) in the Bellenden Ker Range. These mountains are responsible for orographic rain, which reaches recorded annual averages of 100–160 inches in the Babinda-Innisfail-Tully region.

The peculiar topography of this area is also responsible for surge effects which increase the velocities of cyclonic winds. The seemingly erratic pattern of damage—in strips of irregular width, on one or all aspects, although most common on exposed ridges rather than in gullies—is apparently related to topographic features which intensify turbulence. For example, westerly gales passing across the Atherton Tableland, on reaching the lip of the coastal corridor, produce a combination of down-draughts and concentration of wind flow in the valleys. Height of mountains, hills, or other obstacles, speed of the air stream from the west, angle of elevation of the obstacle, and smoothness or roughness of the ground or vegetation surfaces are all considered to influence the extent, speed, and direction of the resulting turbulence eddies, whether horizontal or vertical. Such topographic effects would explain freakish differences in behaviour of the cyclone, e.g. negligible damage at Tully (protected to the west by Mt. Tyson), severe damage at El Arish (lower and different mountain formations to the west), and increased damage along roadways and railway lines, or on ridges and spurs rather than in gullies. Quantitative meteorological data are lacking, but "the tops of ridges facing the wind will experience an increased wind by amounts ranging from 10 to 30 per cent. or more. This would aggravate damage, since the force on an obstacle increases as the square of the wind speed" (E. L. Deacon, personal communication).

The present cyclone, which received the meteorological code name of "Agnes", approached the Queensland coast near Mackay (19–20° S.), veered north-westwards to cross the coast just north of Townsville, and continued in a west-north-westerly direction (see Fig. 1). It reached its highest intensity on March 6 and 7, when most damage was done to the settled areas of coastal north Queensland. As the cyclone lost intensity, torrential rains fell over the headwaters of the Gulf rivers, causing heavy flooding. A feature of ecological importance, however, in certain areas was that the cyclone was "dry", i.e. was accompanied by rain of only moderate intensity (Newman, Martin, and Wilkie 1956).

At Cairns, the wind direction was predominantly from the west, tending to north-west, and finally north. Maximum gusts reached 92 m.p.h. (about midnight of March 6), averaging 50–70 m.p.h., at the height of the storm. Gale force winds, i.e. in excess of 30 m.p.h., occurred within a radius of 800 miles of the centre of the

cyclone, which indicated the extraordinary potential for damage. Easterly gales struck the coast south of Townsville.

In the closely settled region south of Cairns, damage was increased owing to topographic effects of the rugged granitic ranges bordering the lowland corridor. The corridor varies from about 4 to 12 miles wide, is flanked almost continuously by mountains averaging 2000–4000 ft high, and runs for a distance of about 60 miles in a north-south direction between Cairns and Tully (see Fig. 2).

Greatest damage to forests, crops, and buildings was in a relatively narrow strip about 6 miles wide between Codfish and Figtree Creeks, north of Babinda.

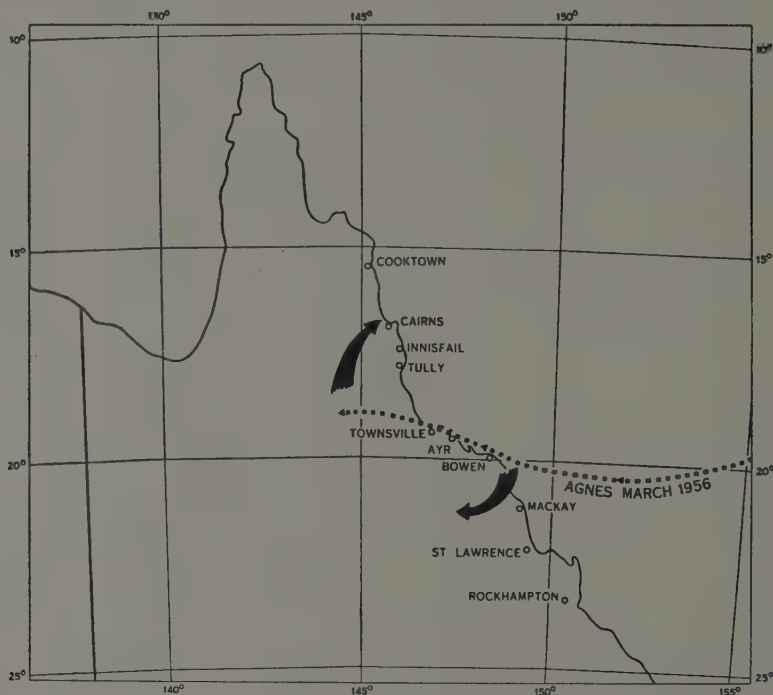


Fig. 1.—Track of Townsville cyclone ("Agnes") which reached its peak in north Queensland on March 6, 1956. Note opposite wind directions in Cairns and Bowen districts.

II. STRUCTURAL AND FLORISTIC REACTIONS OF THE FORESTS

The severity of damage, gauged from the shattering of tall trees and stripping of foliage, varied considerably with topography, i.e. whether the trees were relatively sheltered in valley bottoms, or on exposed slopes. Cyclone-damaged forest appeared from a distance to have been scorched by fire, or to have been ring-barked.

In more sheltered areas (e.g. Happy Valley near Babinda), the rain-forest has an even canopy level averaging 80–110 ft high, with a dense lawyer vine (*Calamus australis* (Mart.) Beccari) understorey about 10 ft high. Here, windthrows and large gaps were uncommon, and damage mostly involved defoliation of tree layers above

12–20 ft high. This sort of damage allows admission of light, favouring the development of *Calamus* spp. and other lianes which are otherwise restricted to well-lit edges of clearings, tracks, or creek banks. The occurrence of a characteristic vine understorey in this type of rain-forest is due to this intermittent defoliation by cyclones. Scattered gaps in the canopy caused by occasional windthrows of tall, unstable trees are rapidly closed by the growth of saplings formerly suppressed by low light intensities, and the result is a forest with a continuous, even canopy with a normal vine cover. The rarity of trees taller than about 100 ft is probably related not only to

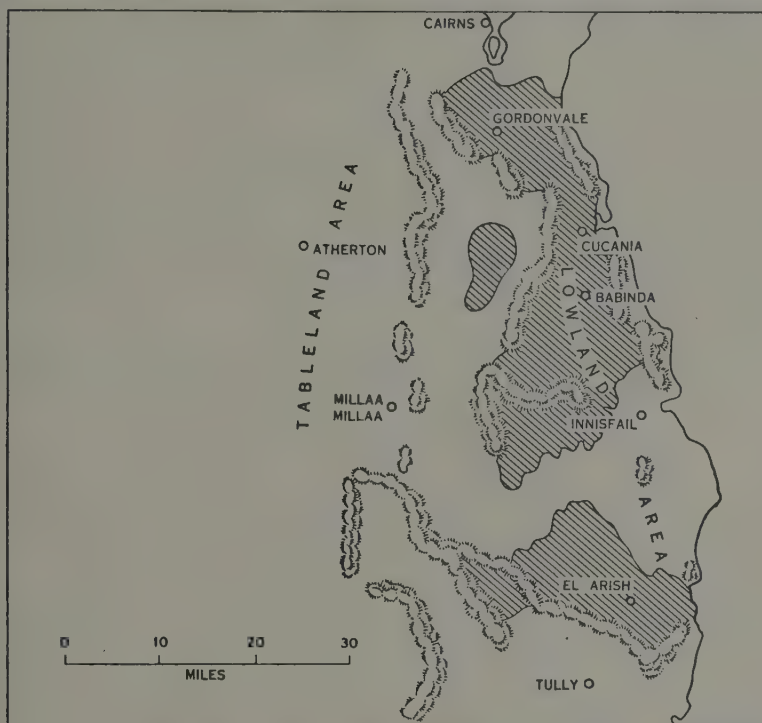


Fig. 2.—Tropical lowland area south of Cairns, north Queensland, showing main mountain ranges with “corridors” which intensify cyclonic turbulence. General areas of cyclone scrubs are shaded, and are particularly well developed on the foothills of ranges west of Cucania, Innisfail (Cooroo), and El Arish.

moderate wind-shearing but also to limited soil aeration, and possibly to the narrow diurnal temperature swing (Webb 1956). This rain-forest is a variety of the tropical subform termed mesophyll vine forest (Webb, unpublished data), and is characterized by a large number of dominant species, three tree layers, prominence of vascular epiphytes and robust lianes in upper tree storeys, prominence of plank buttresses, mesophyll-macrophyll leaf sizes, and local abundance of palms. The soils are relatively deep latosols of colluvial or alluvial origin, from basalt, granite, and schist.

On exposed spurs of the coastal ranges, where localized wind surge and turbulence do not occur, the structure of the rain-forest described above is modified by the appearance of emergent trees, including hickory wattle (*Acacia aulacocarpa* A.

Cunn.), to 60–90 ft above a lower, somewhat uneven canopy 40–60 ft high. An example is the upper western slopes of the Malbon Thompson Range, where fan palms (*Licuala muelleri* Wendl. & Drude) are, with *Calamus* spp., also prominent in the understorey. It seems that the composition of this community is also due to previous cyclone damage. The occurrence of old emergent *A. aulacocarpa* trees with d.b.h.* as large as 36 in., with younger trees of the same species, is characteristic of the rain-forests on shallower, mainly granitic soils of the coastal spurs and ridges, e.g. Dagmar Range and Whyanbeel Creek near Daintree, Claudie River area, and Mt. Mackay and other mountains of the Cairns-Tully lowland corridor. It could be argued that past fires have influenced these forests, since it is well known that fire hastens the germination of *Acacia* seeds. However, there is no evidence that fires have occurred frequently enough to promote the regeneration of *A. aulacocarpa* observed remote from fire-paths and fire sources in adjacent savannah. It is possible that openings in the canopy caused by cyclones, and not followed by fire, may result in the germination of *Acacia* seeds which have been present in the soil for a considerable time. This is supported by indirect evidence that *A. dealbata* Link. seeds in Tasmanian forests of *Eucalyptus regnans* F. Muell. remain viable for about 300 years, which period ensures their germination, without fire, once light is admitted to the forest floor (J. M. Gilbert, personal communication).

A comparison of the effects of fire and cyclones was made possible by a study of the seral changes in a community at Mt. Tyson near Tully, damaged by fire in 1946. This community comprised mixtures of vine and sclerophyll forest species ("bastard scrub"), with *Eucalyptus pellita* F. Muell., *Casuarina torulosa* Ait., and *Tristania conferta* R.Br. in addition to *Acacia aulacocarpa*. Openings were colonized by vine forest or sclerophyll forest pioneers, sedges, and vines. There were marked differences in the liane flora, depending on the extent of fire damage. One of the earliest vines in the succession after fire was *Rubus alceaefolius* Poir., which was frequently followed by *Flagellaria indica* L. On the other hand, species of *Calamus* are fire-sensitive and not associated with *Acacia* in the early stages of succession after fire, although, as suggested above, such an association may result from cyclone damage alone.

Finally, an extremely modified type of rain-forest popularly known as "cyclone scrub" occurs in certain topographic positions, apparently owing to local intensification of wind pressures, if only transiently, during cyclones. In contrast to the forests discussed above, the vine understorey extends upwards as part of the uneven canopy level. The resulting appearance is a dense mass of vines (e.g. *Calamus australis*, *Faradaya splendida* F. Muell., *Haussmannianthes jucunda* (F. Muell.) v. Steenis, *Entada scandens* Benth., *Hypserpa decumbens* (Benth.) Diels, *Omphalea queenslandiae* Bail., and *Ipomoea* spp.), and small trees at all stages of secondary succession 10–40 ft high, with scattered vine-draped emergents to 60–80 ft (see Plate 2). One of the best examples is on the lower slopes of the Bellenden Ker Range behind Cucania. It is locally believed (W. Kerns, personal communication) that emergents such as *Backhousia bancroftii* Bail. & F. Muell. and *Elaeocarpus grandis* F. Muell. never reach maturity. After reaching a certain height, they collapse owing to previous weakening by gales, weight of vines, or accelerated erosion of lithosols on steep granitic slopes.

*Diameter, breast height.

During the March 6 cyclone, few large trees were uprooted, although large branches were commonly broken off, particularly in brittle ("carroty") species such as milky pine (*Alstonia scholaris* R. Br.). In the cyclone scrub areas, saplings and smaller trees (up to about 12 in. d.b.h.) were commonly shattered, and some stems broken off near ground level (see Plate 1, Figs. 1 and 2). The dry weather preceding the cyclone may have been responsible for the rarity of uprooted trees. Uprooting, particularly of shallow-rooted species, is generally associated with gales following continuous rains which have rendered soils sodden and unstable for root anchorage. Such severe damage leaves a mass of broken, leafless trees, and is the cause of the low canopies typical of cyclone scrubs, and of the luxuriant growth of *Calamus* and other vines over the natural trelliswork.

III. PHENOLOGICAL REACTIONS

Throughout the cyclone belt, leaf sprout (Meyer and Anderson 1952) or flushing was beginning on some denuded trees about a fortnight after the cyclone, and eventually became general. Bangalow palms (*Archontophoenix alexandrae* F. Muell.) flowered immediately after the storm; it is possible, as Vaughan and Wiehe (1937) suggested, that flowering of certain species may be "triggered" by cyclones. It is popularly believed that relatively dry years favour flowering of tree species which under normal rainfalls flower only once every 2 or 3 years.

IV. FIRE RISK

When periods of dry, hot, windy weather follow cyclonic damage, fire risk is great, even in mesic tropical rain-forests traditionally regarded as immune to fire. The abundance of fallen branches and suspended dead vines, and of fragmented shrivelled leaves, provides ideal conditions for the entrance of fires from adjacent sugar cane farms. The result is a characteristic pattern of grassland tongues (e.g. with *Acacia aulacocarpa* and *Imperata cylindrica* (J.) Beauv. var. *major* (Nees) C. E. Hubbard as fire indicators) ascending fired spurs, with patches of vine forest in moister gullies. Several local fires entered rain-forest during the dry fortnight following the "dry cyclone" of March 6, 1956—e.g. bordering cane farms near Knowles Creek, Babinda; near Silkwood; and near Maadi (south of Innisfail).

V. RECOVERY OF FORESTS

About 9 months after the March 6, 1956, cyclone, rampant vine growth had obliterated most signs of the previous widespread damage. In typical cyclone scrub areas, the grey stems of scattered emergents were conspicuous, and not yet fully draped by vines. The broken branches of these emergents were coppicing freely. This irregular distribution of tree crown foliage was fairly prominent on mountain spurs, in contrast to less damaged trees in the gullies. Fifteen months later, patches of trees on the upper eastern slopes of Mt. Bartle Frere were moribund and apparently would not recover (J. H. Wilkie, personal communication). In contrast, cyclonic damage in July 1954 to rain-forests of the Kin Kin area, south Queensland, defoliated most species (with a few exceptions such as *Flindersia schottiana* F. Muell.) but practically all trees recovered.

VI. COMPARISON WITH OVERSEAS EXAMPLES

In southern Nigeria, Jones (1955, 1956) and Richards (1955) considered that phases of low or tall "closed scrub" were derived from high forest by tornado damage. These scrubs are characterized by abundant vines and by isolated emergents termed "climber towers". Frequent damage was believed to cause these scrub-climber tangles, whereas intermittent damage would be quickly healed by growth of middle storey trees. Statistical data confirmed the visual impression that middle storey trees suffered most from tornadoes, which tended to recur in the same places (cf. Innisfail area). It was concluded that the best-known forms of west African rain-forest are essentially old secondary forest, damaged also by elephants, and that true primary forest is rare and may have a simpler floristic composition and structure. The cyclone scrubs of north Queensland closely resemble in structure the low closed scrubs of west Africa, and like them, contain saplings and regenerating tree species found in tall, even-canopied lowland rain-forest. These resemblances are the only justification for using the term "scrub" for rain-forest in Queensland, and the term "cyclone (vine) scrub" should be restricted to the communities described above. After the March 6 cyclone, trees of intermediate girth classes averaging 25-50 in. were noticed to be most commonly shattered. It is an interesting conjecture (Jones 1955, 1956) that freedom from disturbance might produce a simplified stable climax forest. This occurs in temperate rain-forest in Tasmania, which has been free from fire for some 500 years (W. D. Jackson and J. M. Gilbert, personal communication), but tropical rain-forest seems to be a different ecological entity. In parts of lowland north Queensland there are patches of forest with simplified tree layers dominated by a few species, e.g. *Tristania pachysperma* at Eubenangee, or *Castanospermum australe* A. Cunn. and *Dysoxylum pettigrewianum* Bail. at Clump Point. While these forests have been subject to variable cyclonic damage in the past, there is some evidence that the simplified composition is associated with unfavourable edaphic factors i.e. low soil aeration at Eubenangee and low soil water availability at Clump Point (Webb 1956).

The "hurricane forest" in the West Indies described by Beard (1945) is apparently a depleted variety of lower montane rain-forest, with palms and tree ferns common. Vines are inconspicuous and the herb layer dense. These forests are found on steep slopes above about 1500 ft elevation. Big trees are scattered among thickets of young growth, forming a variable canopy 30-40 ft above ground. With the notable exception of absence of vines and tree ferns, the hurricane forests are structurally related to the north Queensland cyclone scrubs. They have little resemblance to submontane-subtropical forests in Queensland or New South Wales, where repeated disturbance by cyclones or fire is believed to produce savannahs (Herbert 1938; Baur 1957).

In Kelantan, Malaya, a limited area of forest was "clear-felled" by an unusually severe hurricane, probably associated with the Krakatau eruption of 1883. The resulting "storm forests" had several distinctive features, including reduction of floristic variety, reduction of storeys to two, abundance of lianes, absence of giant trees, tendency to dominance by one or two species among the large trees, and high proportion of spongy heartwood owing to excessively fast growth (Browne 1949; Wyatt-Smith

1954). This forest has some similarities to patches of rain-forest at Clump Point and Tolga, north Queensland. Cases have been recorded where tall, mature rain-forest was almost completely levelled in a broad strip, e.g. at Mission Beach and Clump Point in 1918, and at Yabba, near Urbenville, N.S.W., on February 20, 1954. As with fire, this damage by cyclone may result in even-aged stands of dominant trees.

VII. DISCUSSION

The rain-forests of the north Queensland lowlands and foothills, as has been shown, have not escaped severe or general cyclone damage for periods longer than 3–40 years, although this damage varies in intensity in different places. Farther south along the eastern Australian coast, cyclone damage is more sporadic and local. Freedom from gross disturbance for a period of time longer than the life span of the longest-lived individuals is necessary for a community to reach maturity (Beadle and Costin 1952). No precise data are available, but a reasonable estimate of the age of mature tropical-subtropical rain-forest dominants would be at least 300 years (cf. Francis 1928). It is unlikely that tropical rain-forest in a given area in Queensland would be immune from cyclone damage for the periods of about 500 years or more necessary to develop and maintain a climax forest.

In west Africa it has become increasingly difficult to separate primary and advanced secondary rain-forest (Richards 1952, 1955; Jones 1955); while Vaughan and Wiehe (1937) believed that "cyclones have played a significant part in the evolution of the climax forests" of Mauritius. The concept of a stable climax forest in eastern North America is criticized by Raup (1956), since natural catastrophes (including windthrows and fire) occur within the life span of most trees in this region.

Present observations in north Queensland suggest that cyclones are a potent ecological factor which regularly upsets forest equilibrium, with far-reaching consequences for the regeneration, suppression, and reproduction of species. The heterogeneity of the tropical biological environment, preserved by cyclones, has important implications for speciation (cf. Dobzhansky 1950). Aubreville's mosaic theory of regeneration (Richards 1952) has also thrown into doubt the concept of a stable tropical forest climax.

In north Queensland, the structural reactions of forests to cyclone damage are of silvicultural concern. Distortion of growth form, random damage to desirable girth classes and saplings, smothering of regeneration by vines and weed trees, fire risk, subsequent parasite attack of damaged trees, and extraction difficulties are all undesirable complications for the forester. Because of the frequency of cyclones, timber crops with rotations of 60 years or more (e.g. *Flindersia brayleyana* F. Muell.) have an uncertain silvicultural future in the tropical lowlands of the Queensland cyclone belt.

VIII. ACKNOWLEDGMENTS

The Deputy Director (Mr. B. W. Newman) of the Commonwealth Meteorological Bureau, Brisbane, freely provided relevant details. The Secretary, Queensland Forestry Department, kindly provided the photograph of "cyclone scrub" near Tully. Mr. W. T. Jones, Field Botanist, C.S.I.R.O., is thanked for the other photographs obtained during a joint field trip.

IX. REFERENCES

- ANON. (1940).—"Results of Rainfall Observations Made in Queensland." (Bur. Met. Aust.: Melbourne.)
- BAUR, G. N. (1957).—Nature and distribution of rain-forests in New South Wales. *Aust. J. Bot.* **5**: 190–233.
- BEADLE, N. C. W., and COSTIN, A. B. (1952).—Ecological classification and nomenclature. *Proc. Linn. Soc. N.S.W.* **77**: 61–82.
- BEARD, J. S. (1945).—The progress of plant succession on the Soufriere of St. Vincent. *J. Ecol.* **33**: 1–9.
- BROWNE, F. G. (1949).—Storm forest in Kelantan. *Malay. Forester* **12**(1): 28–33.
- BRUNT, A. T., and HOGAN, J. (1956).—The occurrence of tropical cyclones in Australian region. *Proc. Trop. Cyclone Symp.*, Brisbane, 1956. Pap. No. 1. (Bur. Met. Aust.: Melbourne.)
- DOBZHANSKY, T. (1950).—Evolution in the tropics. *Amer. Scient.* **38**(2): 209–21.
- FRANCIS, W. D. (1928).—The growth rings in the wood of Australian araucarian conifers. *Proc. Linn. Soc. N.S.W.* **53**: 71–9.
- GABITES, J. F. (1956).—A survey of tropical cyclones in the South Pacific. *Proc. Trop. Cyclone Symp.*, Brisbane, 1956. Pap. No. 2. (Bur. Met. Aust.: Melbourne.)
- HERBERT, D. A. (1938).—The upland savannahs of the Bunya Mountains, south Queensland. *Proc. Roy. Soc. Qd.* **49**: 145–9.
- JONES, E. W. (1955).—Ecological studies on the rain forest of southern Nigeria, IV. *J. Ecol.* **43**: 564–94.
- JONES, E. W. (1956).—Ecological studies on the rain forest of southern Nigeria, IV (contd.). *J. Ecol.* **44**: 83–117.
- MEYER, B. S., and ANDERSON, D. B. (1952).—"Plant Physiology." 2nd Ed. (Chapman and Hall Ltd.: New York.)
- NEWMAN, B. W., MARTIN, A. R., and WILKIE, W. R. (1956).—Occurrence of tropical depressions and cyclones in the Australian region during the summer of 1955–56. *Proc. Trop. Cyclone Symp.*, Brisbane, 1956. Pap. No. 3. (Bur. Met. Aust.: Melbourne.)
- RAUP, H. M. (1956).—In report of meeting, Int. Un. Conserv. Nature, Edinburgh, 1956. *Nature* **178**: 175–7.
- RICHARDS, P. W. (1952).—"The Tropical Rain Forest." (Cambridge Univ. Press.)
- RICHARDS, P. W. (1955).—The secondary succession in the tropical rain forest. *Sci. Progr.* **43**(169): 45–57.
- VAUGHAN, R. E., and WIEHE, P. O. (1937).—Studies on the vegetation of Mauritius. I. *J. Ecol.* **25**: 301.
- WEBB, L. J. (1956).—Environmental studies in Australian rain forest. Parts I–V. Ph.D. Thesis, University of Queensland.
- WYATT-SMITH, J. (1954).—Storm forest in Kelantan. *Malay. Forester* **17**(1): 5–11.

EXPLANATION OF PLATES 1 AND 2

PLATE 1

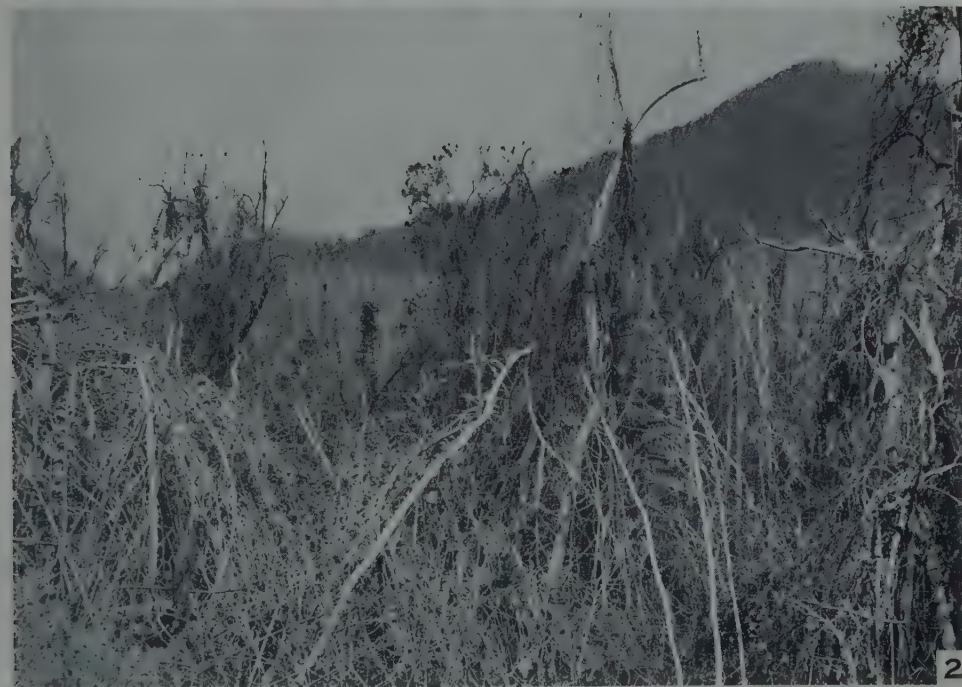
Fig. 1.—Tropical lowland rain-forest damaged by cyclone, March 6, 1956. Note stripped leaves and snapped-off stems of trees with dense tangle of lianes in understorey. Whoopen Creek near Innisfail, north Queensland.

Fig. 2.—Secondary tropical lowland rain-forest damaged by cyclone, March 6, 1956. Leaves mostly stripped, and many branches and smaller trees shattered. The interlaced fallen limbs form a trellis for rampant vine growth, producing cyclone scrub. Fire risk is evident. McNamee Creek near Innisfail, north Queensland.

PLATE 2

Cyclone scrub at Ongera, near Tully, north Queensland. Tropical lowland rain-forest disrupted by frequent cyclones. Note irregular upper canopy level and scattered large trees ("climber towers") abundantly draped by lianes, among which *Calamus* spp. are characteristic.

CYCLONES AS AN ECOLOGICAL FACTOR



CYCLONES AS AN ECOLOGICAL FACTOR



A MONOGRAPHIC STUDY OF *HELICHRYSUM* SUBGENUS *OZOTHAMNUS* (COMPOSITAE) AND OF TWO RELATED GENERA FORMERLY INCLUDED THEREIN

By NANCY T. BURBRIDGE*

[Manuscript received April 8, 1958]

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Summary

The species included under *Helichrysum* sect. *Ozothamnus* in Benthams "Flora Australiensis", vol. 3 (1867), are considered and the status of the section changed to that of subgenus. Two species are reinstated in the monotypic genera *Acanthocladium* and *Argyroglossis* and the remainder retained in *Helichrysum* subgenus *Ozothamnus* and grouped into two sections which also include the more recently described species. All species are fully described.

Section *Hebelaena*, in which the plants are weakly woody perennials growing seasonally from the butt and bearing the capitula in open inflorescences, includes seven species.

Section *Ozothamnus* includes woody species of corymbose or columnar habit of branching and bearing the capitula in dense corymbose inflorescences terminal to the seasonal growth. The section includes 36 species, of which three are new. Two varieties are raised to specific status and a number of taxa treated at the subspecific level.

I. INTRODUCTION

Ozothamnus R. Br. was included as a section under *Helichrysum* by Benthams in the "Flora Australiensis", vol. 3 (1867), where the arrangement to some extent followed that used by A. P. Decandolle in the sixth volume of the "Prodromus" published in 1838. Since then some authors, such as Hooker in his "Flora Tasmaniae" (1856) followed by Rodway in the "Tasmanian Flora" (1903), have preferred to retain Robert Brown's genus but most Australian writers have accepted Benthams lead. Since 1867 a few new species have been described and recently Wakefield (in Vict. Nat. 68: 49 (1951)) has treated the Victorian representatives. This last contribution left the position of some species, particularly those from

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other parts of Australia, in an uncertain state and it was clear that further species remained to be described. An attempt is made here to treat all of them together in order to clarify some nomenclatural points and to describe those without names.

Brown's original description was published in the Transactions of the Linnean Society (12: 125 (1817)) and the wording fits most closely to the woody shrub species though, as was noted by Bentham, he seems to have meant to include certain semi-herbaceous perennials as well. Thus on the specimen of *Helichrysum ramosum* at the British Museum (Natural History) the generic name has been written in by Brown and thus can be linked with a manuscript specific epithet (for a Western Australian specimen) included in his notes.

Two isolated species, *H. dockeri* and *H. argyrolottis*, were added to *Ozothamnus* by Bentham but these have characters, particularly in the pappus, that set them apart from *Helichrysum* and it therefore seems advisable to reinstate them under the two monotypic genera to which they were originally assigned, i.e. to *Acanthocladium* F. Muell. and *Argyrolottis* Turcz. respectively.

The other species described in the "Flora Australiensis" can be placed in two groups. In the first there are a number of semi-herbaceous perennials most of which have paniculate inflorescences, and in the second the plants are perennial woody shrubs with corymbose inflorescences. It is the latter group which agrees most closely with Brown's original diagnosis and they include the Australian species mentioned in his text though Brown did not make the necessary combinations under *Ozothamnus*. These species are further discussed in the taxonomic section below, where reasons are given for the selection of one as the subgeneric lectotype.

In the "Prodromus" Decandolle places the semi-herbaceous species under his Section *EuHelichrysum*, series *Argyraea*, subseries *Hebelaena* and *Antennariiformia*, in which four of the five species listed are Australian. All other species in his series *Argyraea* are non-Australian, many of them being African. In contrast to this woody species are described under the genera *Cassinia*, *Swammerdamia*, and *Ozothamnus*. There is no doubt that though the two groups of species, i.e. the semi-herbaceous and the woody shrubs, differ in some characters they stand together in relation to the other species within *Helichrysum*. These characters do not seem adequate to separate the two groups of species at generic level but subgeneric status does appear appropriate. Furthermore, such treatment of the Australian *Helichrysum* spp. is consistent with that adopted in other countries. Accordingly *Ozothamnus* is here treated as a subgenus with two sections, a re-defined *Hebelaena* to include the semi-herbaceous species and section *Ozothamnus* for the woody shrubs.

Part of the study on which these conclusions were based was carried out while the author was working at the Herbarium of the Royal Botanic Gardens at Kew, and the remainder has been done since her return to Australia. During the latter period the whole paper was greatly expanded by the inclusion of full descriptions of all species. Consequently, while it may be understood that much of the detail is based on material seen at Kew and at the British Museum (Natural History), the actual specimens seen at those institutions are not always quoted. Photographs of the more important Kew sheets are held in the herbarium of the Division of

Plant Industry, C.S.I.R.O., Canberra, in both microfilm negative and enlarged prints. Some of these have been referred to in the text. On the other hand no attempt has been made to record in full detail all the specimens available in Australian herbaria but many sheets have been personally checked and are locally available for consultation. In other species descriptions, abbreviations for the various herbaria are given in accordance with the list published in the "Index Herbariorum", third edition (1956).

So far as the species described in the "Prodromus" are concerned the opinions expressed in this paper are, in almost all cases, based on critical examination of fragments and photographs forwarded to Kew through the courtesy of Sir Edward Salisbury, at that time Director of the Royal Botanic Gardens, and of Prof. Dr. C. Baehni, Director, Conservatoire et Jardin Botanique, Geneva. Additional prints of these photographs are held at Canberra.

II. DISTRIBUTION

So far as is known from the few available specimens *Acanthocladium* is restricted to the Darling River area, having been collected by Beckler with the Victorian Exploring Expedition. The habitat is given as "sandhills". *Argyroglossis* is a plant of the drier areas of the northern parts of the South West Province and the Eremaea in Western Australia. It has been recorded from Lake Hope in the Wongan Hills area but the exact locality for the type is unknown.

The distribution of the species of *Helichrysum* in sect. *Hebelaena* is limited to two widely separated areas. *H. cordatum* and *H. ramosum* are found on the coastal sands of south-west and southern Western Australia. The remainder of the group (*H. obovatum*, *H. whitei*, *H. eriocephalum*, *H. bidwillii*, and *H. vagans*) are found in the north coast areas of New South Wales, the McPherson Range in Queensland, and northward along the coastal ranges to the Mackay area. They seem to be associated with the margins and clearings of rain-forests, a type of locality entirely different from that of the Western Australian species. Through *H. rufescens*, which is found in south-eastern Queensland and northern New South Wales, this series is linked with the woody species of *Ozothamnus* sens. str., but there being no similar linkage for the Western Australian pair of species there is no indication whether these have had a separate origin or whether they represent, with the others, two outliers of a formerly much more widely distributed type.

Among the species of *Ozothamnus* in the narrower sense two main forms may be noted, both of them being represented also in *Hebelaena*. In one the involucre bracts are erect or incurved, the laminae of the innermost being similar to the outer ones and not radiating. In the other there is a considerable difference between the inner and outer bracts and the former bear more or less radiating laminae distinctive in texture. The species grouped under the first of these types are, with one exception, restricted to the Australian mainland. They extend from arid areas in Western Australia, across Victoria, and northwards along the western slopes, south coast, and tablelands of New South Wales into south-eastern Queensland.

Helichrysum obcordatum, which is found in Tasmania, is represented on the mainland by a subspecific taxon which extends through Victoria to New South Wales. None of the species is associated with high-rainfall habitats; all of them appear to occur in arid or open forest sites, though here again *H. rufescens* is exceptional.

The final group, i.e. those with radiating inner involucre bracts, is mainly restricted to high elevations on the southern parts of the Dividing Range in New South Wales, to high elevations or the wetter parts of the Victorian coast, and to Tasmania. In New South Wales the main assemblage of species grades out north of the Australian Alps but *H. dendroideum* and *H. argophyllum* extend further north along the wetter parts of the ranges as far as Point Lookout, on the New England Tableland. According to Wakefield (Vict. Nat. 68: 49 (1951)) *H. dendroideum* also reaches South Australia. The distribution of individual species can be more easily dealt with under each one rather than here but it may be noted that in several cases there is evidence that some form complexes whose variation is ecotypic in expression. This may well be the case in the series *H. dendroideum-argophyllum-conditum*, with its approach to *H. secundiflorum* through the last named and to *H. stirlingii*. Another series is formed by *H. gunnii* in its two (or possibly more) varieties, and yet a third is the complex under *H. ledifolium* in Tasmania with *H. alpinum* on the mainland.

It was hoped that some light could be thrown on these matters by examination of the cytology but up to date the results have been unhelpful.

Species belonging to other sections of *Helichrysum* have not been specially studied and, consequently, comment on the possible relationships, that might be indicated by morphological similarities, cannot be attempted.

III. TAXONOMY

As has been mentioned above it is considered that the two genera *Acanthocladium* F. Muell. and *Argyroglossis* Turcz. are distinct from *Helichrysum* and should not be included in the subgenus *Ozothamnus*. In both, the pappus bristles are distinctly fused at the base and in the latter in particular, bristles continue in clusters above this basal union. The capitula are solitary and in general habit the plants are unlike those included in *Helichrysum*, particularly in *Ozothamnus*. They can be separated with the aid of the following key:

- A. Pappus bristles fused into a basal ring and their cohesion in small clusters of bristles continued higher. Heads solitary.
- B. Involucral bracts not radiating. Heads lateral or axillary (?), heterogamous. Receptacle flat *Acanthocladium*
- BB. Inner involucre bracts radiating. Heads terminal, homogamous. Receptacle conical *Argyroglossis*
- AA. Pappus bristles falling singly or fused into a very short and temporary ring only. Heads clustered in corymbose or paniculate inflorescences *Helichrysum* subgen. *Ozothamnus*
- C. Weakly woody perennials with slender subherbaceous stems. Involucre bracts commonly reflexed and persistent at post anthesis sect. *Hebelaena*
- CC. Woody shrubs with rigid stems. Involucre bracts not or very rarely reflexed but commonly deciduous with the achenes sect. *Ozothamnus*

Acanthocladium F. Muell., *Fragm.* 2: 155 (1861); *Helichrysum* sect. *Ozothamnus* Benth., *Fl. Austral.* 3 (1867) pro parte.

Mueller's genus was based on a single species for which no near affinities have since been described. A new description of this is given below.

Acanthocladium dockeri F. Muell., *Fragm.* 2: 156 (1861); *Helichrysum dockeri* (F. Muell.) Benth., *Fl. Austral.* 3: 626 (1867).

Holotype.—Sandhills near the Darling R., Dr. Beckler, 28.x.1860 (MEL).

Shrub with spinose branchlets and a thick woody butt producing woody shoots. *Leaves* oblong-lanceolate, 7–12 mm long, grey-hoary with soft tomentum similar to that on the branchlets. Ultimate branchlets leafless and terminating in short glabrous pungent apices. *Capitula* solitary, sessile and either axillary or lateral to the upper branchlets. *Involucres* cylindrical at first but later turbinate, 8–10 mm long; outer bracts strongly woolly tomentose on the back but with glabrous straw-coloured membranous or scarious apices; inner bracts longer, the scarious points erect but often acute or apiculate instead of obtuse. *Outer florets* female and very slender. *Pappus* bristles numerous, fused into a basal ring, barbellate towards the ends but almost bare below. *Achenes* minutely papillose. (Fig. 1, B–B₄.)

The Kew sheet of this species is labelled as collected at "Bambamero" and may be regarded as from the type locality if not an isotype. Beckler, having resigned early in October, was left at the base camp in Menindie when Burke and Wills with their party moved north on October 19, and during the following 10 days he was engaged in helping to move stores to a new camp about 7 miles away at "Bambamero", the junction of a creek with the Darling River. The exact period of this work is not clear but as there were complaints about the slowness with the available camels it must have lasted until the 28th. The place is believed to be the junction of the creek joining the Lake Pamamaroo of modern maps to the river. Beckler was again in this area in the following January but the close agreement between the specimens makes it most unlikely that two collecting periods are involved.

Argyroglottis Turcz. in *Bull. Soc. Nat. Moscou* 24(2): 83 (1851); *Helichrysum* sect. *Ozothamnus* Benth., *Fl. Austral.* 3 (1867) pro parte.

This genus also is monotypic and a full description of the single species is given below.

Argyroglottis turbinata Turcz. in *Bull. Soc. Nat. Moscou* 24(2): 84.t.l. (1851); *Helichrysum argyroglottis* Benth., *Fl. Austral.* 3: 626 (1867); *H. turbinatum* (Turcz.) C. A. Gardner, *Enum. Pl. Austral. occ.* 134 (1931) non W. V. Fitzg. in *J. W. Aust. Nat. Hist. Soc.* 1: 23 (1907); *Conanthyrium drummondii* A. Gray in *Hook., Kew J.* 4: 273 (1852).

Holotype.—Swan R., *Drummond Coll.* V. suppl. No. 63 (isotype at Kew).

Shrub or subshrub of slender habit, the stems tomentose when young but the tomentum falling to reveal minute glandular hairs which in their turn are lost from older portions. *Leaves* linear-oblong to oblanceolate, obtuse, asperulous above,

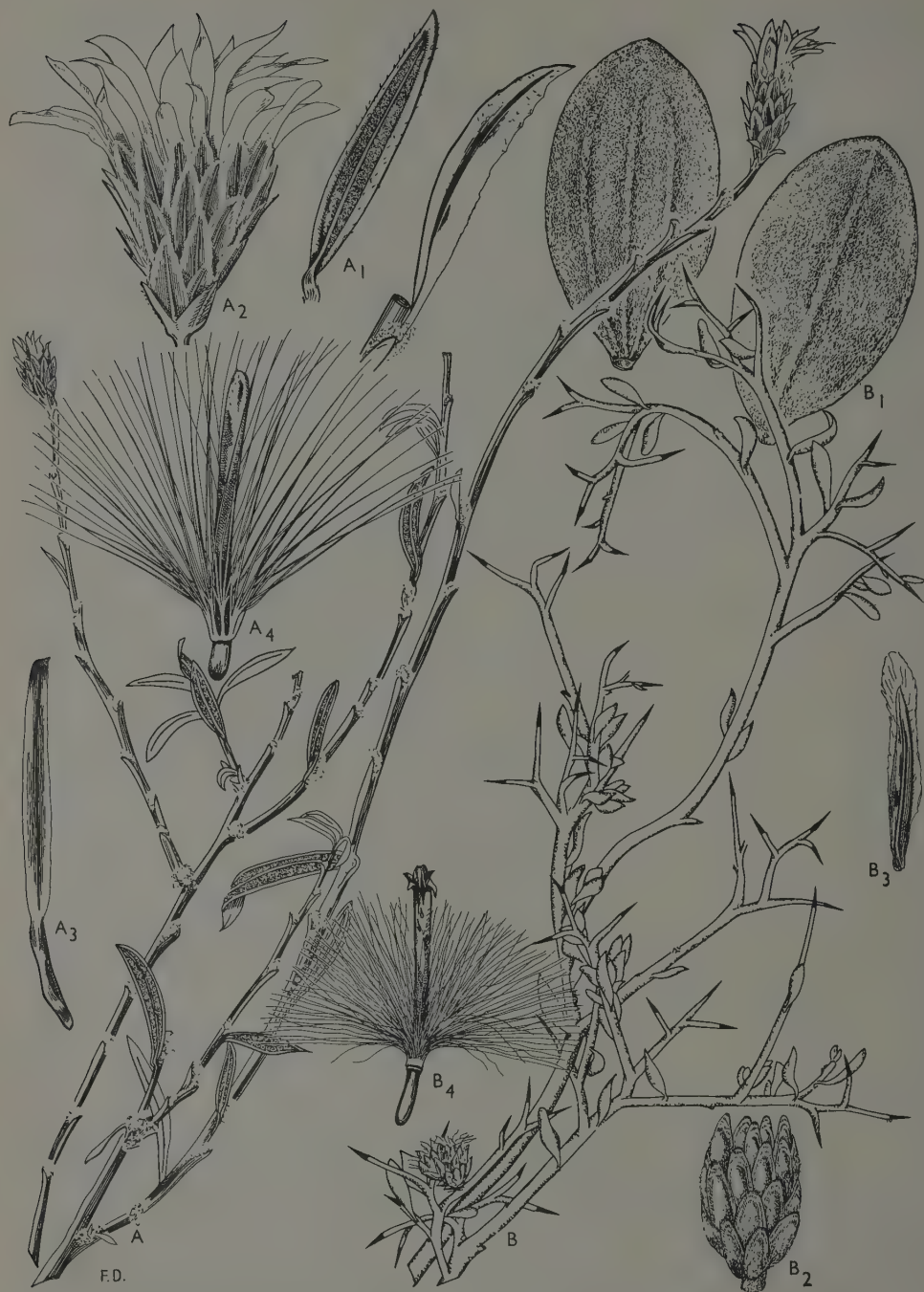


Fig. 1.—A-A₄, *Argyroglottis turbinata* Turcz. B-B₄, *Acanthocladium dockeri* F. Muell. (drawn from holotype). A, small branch (natural size); A₁, upper and lower surfaces of leaf ($\times 3$); A₂, capitulum ($\times 3$); A₃, inner involucre bract ($\times 5$); A₄, floret ($\times 5$). Lettering under B-B₄ follows the same system.

webby-tomentose below, 1–2 cm long, 1·5–4 mm broad, shortly petiolate and woolly in the axils, half clasping and slightly decurrent. *Capitula* solitary, terminal, pyriform-acuminate when young, turbinate later, sessile or almost so, 2 cm long (radiating bracts excl.) subtended by 2–3 short foliage leaves. *Involucral bracts* multiseriate, chartaceous; *outer* ones broad lanceolate to ovate-lanceolate and with a dark centre marked by a distinct midrib and pale margins, densely woolly tomentose when young but later glabrescent with the exposed surface minutely glandular pubescent; innermost bracts narrow, linear, glabrous with a white radiating but obtuse-linear lamina almost or quite as long as the claw. Receptacle conical, florets all hermaphrodite. *Achenes* (immature on specimens studied) apparently angular, the pappus soon deciduous and its loss revealing a small apical umbo; bristles almost free from barbels except towards the apices, joined in a ring at the base but the cohesion continued for clusters of bristles above. After falling the pappus ring splits into these tufts of which the connate bases are reflexed or half coiled. (Fig. 1, A–A₄.)

Specimens Seen.—WESTERN AUSTRALIA: Swan R., *Drummond* 3/1850; Lake Hope, *W. E. Blackall* No. 1259, 2.xi.1931; Wongan Hills, *A. Morrison*, 7.x.1903; Mollerin salt lakes, *W. E. Blackall* 3494, October 1937.

Except for its perennial shrubby habit and the radiating laminae of the inner involucral bracts this species in a mature state bears a superficial resemblance to some of the larger headed species of *Pterigeron*. The character of the capitula and the nature of the pappus leave it without any obvious affinity under *Helichrysum* though in some species there is a similar tendency for the bristles to break away in groups. Where this is so, i.e. in *H. leucopsidium*, the coherence is very short and the pappus is far more persistent than in *Argyroglossis*. Turczaninow failed to find any tails to the anthers but these were noted by Bentham (loc. cit.) and have been observed by the author.

***Helichrysum* subgenus *Ozothamnus* stat. nov.**

Ozothamnus R. Br. in Trans. Linn. Soc. 12: 125 (1817); DC., Prod. 6: 164 (1838); Hook. f., Fl. Tasm. 1: 200 (1856); Rodway, Tasm. Fl. 88 (1903); *Helichrysum* sect. *Ozothamnus* (R. Br.) Benth., Fl. Austral. 3: 625 (1867); *Petalolepsis* Cass. in Bull. Soc. Philom. Paris No. 134 (1817); *Swammerdamia* DC., Prod. 6: 164 (1837).

Section ***Hebelaena*** (DC.) emend.; subseries *Hebelaena* DC., Prod. 6: 180 (1838) pro parte; *Antennariformia* loc. cit. 181; sect. *Ozothamnus* Benth., Fl. Austral. loc. cit. pro parte.

Lectotype.—*H. cordatum* DC., loc. cit.

Herbaceous perennials or subshrubs with weakly woody erect or ascendent branches. The seasonal shoots basal and dying back after flowering but in later growing periods producing branches of the second and third order which behave similarly. *Inflorescences* of clusters of small heads either at the ends of slender almost leafless branches of a very open panicle or terminating the branchlets. *Receptacle* more or less flat and often pitted. *Involucral bracts* more or less tomentose,

the innermost with glabrous tips which may be minute or forming radiating laminae. *Bracts* not deciduous with the achenes but becoming reflexed and commonly persistent. *Florets* all hermaphrodite or commonly with a few outer ones female. *Pappus* soft, barbellate.

Under his subseries *Hebelaena* Decandolle described the species *Helichrysum cordatum*, *H. obovatum*, and *H. populifolium*. Of these the last is South African and has been made the type of a subgeneric taxon by Moesser (Engl. Bot. Jahrb. 44: 289 (1910)). It is quite distinct from the Australian species. Under *Antennariformia* Decandolle described *H. ramosum* and *H. gracile*, which were merged by Bentham in the "Flora Australiensis". Both the subseries were placed under series *Argyraea* by Decandolle, but all the other species he described in the latter are non-Australian. Both the subseries are loosely defined and neither has been taken up by subsequent workers. The word *Hebelaena* seems to have its origin in *hebeo* (= dull) and *laena* (= cloak or mantle) and to refer to the tomentum of the three original species. This character is less evident in *H. ramosum* and in more recently described species.

Section **Ozothamnus** (R. Br.) Benth., Fl. Austral. 3: 625 (1867) pro parte.
Ozothamnus R. Br. in Trans. Linn. Soc. 12: 125 (1817) sensu stricto.

In the remarks associated with his generic description Brown mentions four species for none of which he actually makes the necessary new combination. These species are *Calea pinifolia* Forst. f., *Eupatorium ferrugineum* Labill., *E. rosmarinifolium* Labill., and *Chrysocoma cinerea* Labill. He states that Forster's material though represented in both the Lambert and Banks collections is far from perfect, and his text can be read to suggest that, in his mind, it was the Australian and Tasmanian plants (with which he was familiar in both the field and the herbarium) that formed the nucleus of his new genus.

Furthermore, Hooker, in "Florae Novae Zelandiae" (1: 134 (1853)), also comments on the poorness of the Forster material. He states: "*Ozothamnus pinifolius* Br. (*Calea pinifolia* Forst.) is only known through a very indifferent specimen in Forster's Herbarium, to which the habitat of New Zealand is marked by that author with a mark of doubt. As it has been found by no succeeding collector, I am inclined to suspect it to be more probably a New Caledonian plant. It may be recognized by its very narrow acerose leaves, spreading on all sides and its scarred branches, exactly like those of a pine." Again in the "Handbook to the New Zealand Flora" 145 (1867) Hooker writes (under *Cassinia*) "*C. pinifolia* is a native of New Caledonia and not of New Zealand" but as there is no reference to Forster the correlation is not certain. There is no reference to the species in Guillaumin's "Flore de la Nouvelle-Caledonie" (1948) and its identity seems doubtful. Under these circumstances Forster's name cannot be regarded as eligible for selection as lectotype.

Of the other species mentioned by Brown, *Eupatorium ferrugineum* cannot be directly referred to *Helichrysum* because of the resulting homonymy and it may be doubted whether Brown was entirely clear as to the true identity of *Chrysocoma cinerea* (see notes under *H. gunnii* below). Consequently the remaining species has been selected as the lectotype. It agrees closely with Brown's generic description

and the type sheet has recently been critically discussed by Wakefield (Webbia 9(2): 466-8 (1954)). It is also the only valid specific epithet included under a brief generic diagnosis in Brown's manuscript notes.

Lectotype.—*H. rosmarinifolium* (Labill.) Steud. ex Benth.

Woody shrubs, commonly with short internodes and small leaves. *Capitula* in dense clusters or subumbellate corymbose inflorescences, either terminal to the main stems or at the ends of short laterals and forming compound leafy panicles. *Receptacle* flat or slightly convex. *Involucral bracts* more or less tomentose or glabrescent, the innermost with laminae similar to the outer or radiating and commonly all or most of them deciduous with or soon after the achenes. The claws of the involucral bracts commonly bear golden globular hairs which appear like drops of exudate under the lens. *Florets* all or all except a very few outer ones hermaphrodite. *Pappus* soft and barbellate, sometimes thickened towards the apices, usually remaining attached to the achenes.

The species of the two sections can be identified by the following key.

KEY TO THE AUSTRALIAN SPECIES OF *HELICHRYSUM*, SUBGENUS *OZOTHAMNUS*

- A. Herbaceous or subherbaceous perennials with weakly woody stems which having flowered die back and produce laterals of secondary and tertiary orders in later periods of growth. Bracts commonly reflexed after flowering (section *Hebelaena*)
- B. Capitula clustered at the ends of the slender branches of an open almost leafless panicle
- C. Inner involucral bracts with conspicuous radiating laminae. (Western species)
 - D. Plants densely tomentose except on the upper surfaces of the leaves. Laminae of bracts acute 1. *cordatum*
 - DD. Plants glabrescent, leaves linear to oblanceolate, scabrid above, tomentose or glabrescent below. Laminae of bracts obtuse 2. *ramosum*
- CC. Inner involucral bracts not radiating. Tomentum variable. (Eastern species)
 - E. Leaves obovate, 2-3 cm long, slightly woolly or glabrescent below 3. *obovatum*
 - EE. Leaves linear- or elliptical-lanceolate, 5-9 cm long, densely arachnoid-tomentose below 4. *whitei*
- BB. Capitula clustered at the ends of leafy branches lateral to the previous series or in terminal leafy corymbose panicles
 - F. Inner involucral bracts radiating, outer bracts and both surfaces of leaves densely arachnoid-tomentose. Florets above 40 5. *eriocephalum*
 - FF. Inner bracts not radiating, outer ones with few webby hairs or almost glabrous
 - G. Plants thinly arachnoid-tomentose, leaves ovate, oblong or oblanceolate. Florets about 20 per head. Branchlets more or less striate 6. *bidwillii*
 - GG. Plants almost glabrous except for the minutely scurfy tomentum of the undersides of the leaves. Florets about 12 per head. Branchlets angular .. 7. *vagans*
- AA. Woody shrubs, not producing seasonal shoots which die back after flowering. Heads in dense clusters at the ends of leafy branches or in corymbose or paniculate inflorescences. Bracts not or only very rarely reflexed after flowering, the inner ones often deciduous with the achenes (section *Ozothamnus*)
- H. Involucral bracts erect or incurved, either thin and scarious or chartaceous, straw-coloured or milky white. The tips of the innermost similar in texture to most of the outer ones (see also *H. scutellifolium* below)
- I. Capitula forming pyramidal inflorescences. Bracts straw-coloured or rufescent. Leaves never decurrent
- J. Leaves flat or slightly recurved. Capitula campanulate, bracts scarious and thin 8. *rufescens*

- JJ. Leaves linear, revolute. Capitula cylindrical, bracts thickened above, scarious-chartaceous below9. *cassinoides*
- II. Capitula in broad corymbs or subglobose clusters
- K. Leaves erect or spreading, never all reflexed and minute
- L. Decurrent tissue on stem, if present, due to partial adherence of leaves, the ridges not (except in *H. blackallii*) reaching fully from node to node
- M. Leaves narrow-linear, revolute, more than 7 mm long, not partially attached to the stem, which bears no decurrent tissue
- N. Bracts milky white or pinkish. Leaves 7-15 mm long, stems thinly tomentose10. *diosmifolium*
- NN. Bracts scarious, pale. Leaves 20-30 mm long, stems densely tomentose11. *kempei*
- MM. Leaves often partially adherent, if more than 7 mm long then attached for at least half their length
- O. Leaves free from the stem except for the divergent stem-clasping bases (when developed). Achenes papillose or papillose-hairy
- P. Bases of leaves not auriculate. Bracts milky white12. *tuckeri*
- PP. Bases of leaves auriculate. Bracts scarious
- Q. Leaf auricles rounded and almost helicoid. Capitula with about 30 florets13. *diotophyllum*
- QQ. Leaf auricles narrow and becoming more or less horizontally divergent as stem diameter increases. Capitula with up to 16 florets14. *cassiope*
- OO. Leaves not broadened at base, decurrent on stem and adherent for about half their length, the decurrent lines not or almost extending to the node below. Achenes hairy
- R. Leaves 12-20 mm long. Capitula with 30-40 florets. Bracts scarious and straw-coloured15. *tesselatum*
- RR. Leaves 3-8 mm long. Bracts opaque, pale or milky white
- S. Leaves attached for less than half their length. Capitula 18-22-flowered16. *adnatum*
- SS. Leaves attached for more than half their length. Capitula 12-14-flowered17. *blackallii*
- LL. Stems with lines of decurrent tissue from node to node, commonly with a constriction or minute petiole at base of free portion of blade
- T. Leaves linear, revolute
- U. Leaves retuse with reflexed apices, the decurrent lines narrow and with tomentose strips between. Constriction at base of blade often obscure18. *bilobum* and subsp.
- UU. Leaves obtuse, truncate or apiculate, the decurrent tissue thick and almost obscuring the tomentum between
- V. Involucral bracts scarious and straw-coloured, with membranous margins. Leaves much wrinkled when dry, with conspicuous constriction at base19. *catadromum*
- VV. Involucral bracts stiff, milky white. Leaves scabrid, with slight constriction at base20. *occidentale*
- TT. Leaves obcordate to obovate, flat or slightly recurved. Decurrent tissue lines often partially obscured by tomentum21. *obcordatum* and subsp.
- KK. Leaves minute, orbicular, revolute, all reflexed. Decurrent lines sometimes evident on longer internodes. Bracts milky-opaque, often crumpled or the outer ones pale22. *lepidophyllum*
- HH. Involucral bracts erect or spreading, the tips of the innermost differing in texture from those of the outer, scarious or opaque, often crumpled, dilute or milky white, rarely purplish

- W. Innermost bracts with pale or white apices
- X. Leaves at least 5 mm long. Capitula shortly pedunculate
- Y. Laminae of the innermost bracts minutely fimbriate, not crumpled. Leaves coarsely linear and commonly marked by transverse or irregular wrinkles
- Z. Leaves 3-5 cm long. Tomentum arachnoid. Laminae of innermost bracts slender and acuminate, thinly membranous to white23. *reticulatum*
- ZZ. Leaves 1.5-3 cm long. Tomentum of crisped woolly hairs. Laminae of innermost bracts obtuse or acute, milky white24. *buftoni*
- YY. Laminae of the innermost bracts not fimbriate though sometimes crumpled or slightly irregular on the margins
- a. Capitula in terminal corymbs or clusters, not forming long floriferous secund sprays along the lateral branches and branchlets
- b. Leaves linear to broad lanceolate, at least 5 times as long as broad
- c. Margins of leaves flat or recurved
- d. Involucres hemispherical to campanulate, more than 50-flowered. Leaves lanceolate to broad elliptical, commonly 3-nerved and more than 6 mm wide25. *stirlingii*
- dd. Involucres cylindroid to narrow turbinate, the florets less than 30. Leaves 2-6 mm wide
- e. Stems densely tomentose, the striations obscure
- f. Outer involucral bracts with thin ragged apices. Leaves elliptical to linear-lanceolate, up to 8 cm long and to 6 mm broad, often obscurely 3-nerved26. *argophyllum*
- ff. Outer involucral bracts with entire pale apices and grading into the laminate inner ones. Leaves narrow-linear and smaller than those of the above27. *conditum*
- ee. Stems angular and soon glabrous but the tomentum persistent on the raised striations. Leaves 1-nerved and often minutely undulate on the margins. Outer bracts with entire or subentire margins, not grading into the innermost laminate ones28. *dendroideum*
- cc. Leaves narrow and revolute on the margins (except in form under *H. gunnii*)
- g. Leaves not antrorsely scabrid. Tomentum webby or woolly
- h. Tomentum grey and arachnoid. Capitula 4-5.5 mm long, 6-12-flowered. Innermost bracts with conspicuous laminae29. *gunnii* subsp. *gunnii*
- hh. Tomentum closely woolly, often stained with yellow exudate especially on the under-sides of the leaves towards their bases. Capitula 6-7 mm long, 16-20-flowered. Innermost bracts with small white laminae, the outer ones with the marginal cilia tangled in resin29a. *gunnii* subsp. *paralium*
- gg. Leaves antrorsely scabrid-muricate. Tomentum of branches thinly woolly. Capitula very numerous in flat-topped corymbs. Outer bracts often purpurascens30. *rosmarinifolium*
- bb. Leaves obovate, cuneate, and very obtuse or if oblong less than 5 times as long as broad
- i. Leaves oblong, thick, recurved or revolute on the margins
- j. Leaves revolute on the margins, the upper surface subglabrous and often subresinous, more or less reflexed at least when old. Tasmanian species complex
- k. Low robust shrubs of high altitudes and mountain tops, the old leaf bases persistent. Capitula 6-7 mm long, 10-14-flowered31. *ledifolium* subsp. *ledifolium*
- kk. Erect shrubs to 2 m high, on lower mountain slopes, plains, or near coasts
- l. Capitula 8-10-flowered. Leaves strongly reflexed or almost coiled especially when old

- m. Leaves 7–20 mm long. Capitula 5·5–6 mm long. Plants of lower slopes31a. *ledifolium* subsp. *purpurascens*
- mm. Leaves 3–10 mm long recurved to reflexed even when young. Capitula 4–5 mm long. Coastal shrubs, the branches often subwhorled owing to their development immediately below the old corymbs
-31b. *ledifolium* subsp. *reflexum*
- ll. Capitula 3·5–4·5 mm long, 5–6-flowered. Leaves 4–7 mm long, recurved when old. Plants of high plains31c. *ledifolium* subsp. *ericifolium*
- jj. Leaves recurved on the margins, subresinous or with appressed webby hairs above and densely tomentose below. Mainland species32. *alpinum*
- ii. Leaves obovate to cuneate, flat or slightly recurved on the margins
 - n. Stems slender. Capitula narrow and more or less cylindrical, in spreading corymbs. White laminae of inner bracts conspicuous. Florets less than 1033. *cuneifolium*
 - nn. Stems rigid and often robust. Capitula turbinate, in short corymbs or subglobose clusters. White laminae of inner bracts often inconspicuous
 - o. Leaves 15–20 mm long, narrowed into slender petioles. Capitula with more than 20 florets34. *antennaria*
 - oo. Leaves 5–12 mm long, without slender petioles. Capitula with about 15 florets35. *backhousii*
- aa. Capitula crowded at the ends of numerous short lateral branches and forming long floriferous sprays which may be secund and pyramidal around the main shoot
- p. Leaves glabrous above and with a close thin and flat tomentum below
-36. *thyrsoides*
- pp. Leaves webby or glabrescent above, thickly tomentose below ..37. *secundiflorum*
- XX. Leaves less than 5 mm long (rarely with a few somewhat longer ones subtending lateral branches). Capitula sessile or almost so, forming subglobose clusters and either terminal or terminating short laterals
- q. Leaves not closely appressed to stem, 2·5–4 mm long, oblong or linear
- r. Leaves spreading or almost reflexed, oblong. Tomentum on stem not webby
 - s. Leaves and stems almost glabrous, slightly resinous. Terminal clusters or capitula sometimes surrounded with whorls of branchlets developed from the upper axils. Laminae of innermost bracts not conspicuous38. *selaginoides*
 - ss. Leaves and stems shortly woolly pubescent, often yellowish. Capitula in dense clusters without subtending branchlets formed during flowering (sometimes developed later). Laminae of innermost bracts conspicuous
 -31c. *ledifolium* subsp. *ericifolium*
- rr. Leaves erect, linear, revolute on the margins but densely webby-tomentose below. Stems webby-tomentose. Laminae of innermost bracts white, erect or slightly spreading39. *expansifolium*
- qq. Leaves 1–2 mm long, adpressed to the white tomentum of the stems
 - t. Leaves erect, ovate-linear, with revolute margins not quite concealing the tomentose midrib. Innermost bracts with spreading white tips40. *hookeri*
 - tt. Leaves orbicular, reflexed so that only the upper surface is visible. Innermost bracts without evident laminae or the laminae minute41. *scutellifolium*
- WW. Bracts commonly dark or purplish especially on their flat spreading apices. Capitula sessile in small globose clusters terminating the branchlets. Leaves linear to oblanceolate, erect and loosely imbricate42. *lycopodioides*

1. *Helichrysum cordatum* DC., Prod. 6: 180 (1838); Benth., Fl. Austral. 3: 627 (1867).

Lectotype.—Barren shores of King George Sound, A. Cunningham (G-DC), 1818 (non vidi) (isotype at Kew?).

Weakly woody perennial with spreading or ascendent-erect and densely tomentose branches. *Leaves* petiolate, cordate, obtuse, 2–6 cm long (basal ones longer), 2–2.5 cm wide; upper surface green but drying olive green or almost black, subglabrous; lower surface densely white-tomentose like the stems and with slightly prominent nerves. *Panicles* large and very open owing to the divaricate branches, either bractless or with subtending much reduced leaf-like bracts. *Capitula* shortly pedunculate and corymbosely clustered at the ends of the branches, turbinate, 4.5–5.5 mm long. *Outer involucrel bracts* tomentose, grading into the *inner bracts*, which have narrow claws and acute spreading white laminae. All bracts spreading or reflexed when over-mature. *Florets* 15–20, the outer few female. *Achenes* glabrous or subpapillose. Receptacle pitted. (Plate 1.)

A plant of the sandhills and sandy soils of the south-west and west coasts of Western Australia. Seasonal shoots are produced from the base and terminate in inflorescences but secondary branches of a similar habit may be produced from the lateral axils in a later surge of growth. The involucrel bracts are persistent and become reflexed when the achenes fall.

With his description Decandolle states “in Nova-Hollandia orientali et ad litt. sterilia orae austr. occid. ad King George-sound . . . (v.s. comm. a Mus. reg. Par. ex itin. Baudin et cl. A. Cunn.)”. Copies of the labels of the relevant specimens at Geneva read: (1) “Nouvelle Hollande cote orient. Mus. de Paris 1821”, and (2) “Nouvelle Hollande, cote orient. Mus. de Paris 1821. barren shores of King Geo. Sound S.W. Coast 1818. 81”. It thus appears that the reference to the east coast is an error, especially since the species is only known from the south-west of Western Australia.

At Kew there is a sheet to which four specimens are attached. Two of these are associated with labels written by Allan Cunningham. One reads “61. *Helichrysum cordatum* DC. Prod. VI. 180 A. Cunningham, *Gnaphalium* Sandy Downs King George’s Sound S.W. Coast, 31. Jan. 1818” and the other “61. *Helichrysum cordatum* DC. Prod. VI. 180, *Gnaphalium* sp. A. Cunningham barren brushy ridges King George’s Sound Dec. 1821”. A photograph of this sheet is held as No. 1585 at Canberra, but from the notes it would appear that though the former specimen may have been collected at the same time it cannot be proved to be isotypic.

Other Specimens Studied.—WESTERN AUSTRALIA: King George Sound, *R. Brown* (Bennett No. 2207) (KEW, BM); King George Sound, 1st voyage of Mermaid, 1818, No. 86, *A. Cunningham*; King George Sound, Voyage of Bathurst, 1821–22, No. 292, *A. Cunningham* (BM); Swan R., *Drummond*, Coll. II, No. 168, 1844 (KEW, BM); Swan R., *Mylne* (KEW); Perth, *Capt. J. Mangles*, July and Aug. 1831; District Swan, *Pritzel* 264, Feb. 1901; Claremont, *C. Andrews*, Feb. 1902 (BM); Swan R. estuary, *Steedman*, Jan. 1922 (PERTH); Geographe Bay, *Mrs. Irvine* (BM, PERTH); King’s Park, Perth, *R. D. Royce* 5382, 10.ii.1956 (PERTH, CANB); Warren Drift Sands *F. M. C. Schoch*, 22.iii.1917 (BM).

2. *Helichrysum ramosum* DC., Prod. 6: 181 (1838); Benth., Fl. Austral. 3: 626 (1867); *H. gracile* DC., Prod. loc. cit.

Holotype.—“A branching plant of apparently annual duration of rare occurrence on the shores of King George’s Sound”—*Allan Cunningham* (G-DC) (non vidi).

Holotype of Synonym.—"dry barren situation King George Sound 1821"—*Allan Cunningham* (G-DC) (isotype at Kew).

Short-lived perennial or herbaceous subshrub of weakly woody spreading or erect stems (sometimes rhizomatous according to Bentham). Primary shoots terminating in panicles and later producing laterals of the second and subsequent orders of branching. *Branches* pithy, glabrous or glabrescent. *Leaves* linear-ob lanceolate or narrow elliptical, narrowed into short petioles, 1.5–2 cm long, 2–4 mm broad, glabrous above, subglabrous or thinly woolly beneath. *Capitula* in dense corymbose clusters terminating the minutely bracteate, widely divaricate panicle branches, 3.5–4.5 mm long, turbinate-campanulate. *Outer bracts* loosely woolly, sometimes purplish; *inner* with conspicuous obtuse white laminae; all spreading and reflexed when over-mature. *Florets* 20–25, a few of the outer ones female. *Achenes* glabrous. *Receptacle* pitted.

The original material at Geneva has not been seen and the quotations of specimen labels are given from copies received.

Specimens Studied.—WESTERN AUSTRALIA: Darling Range, District Wellington, *Pritzel* 194, January 1901 (BM); Margaret R., *W. E. Blackall*, December 1930 (BM, PERTH); Augusta, *A. J. Hall*, Aug.–Jan. 1936; Pemberton, *T. N. Stoate*, 14.xii.1939; Nornalup, *W. E. Blackall*, December (PERTH); Porongorups, *J. H. Maiden*, Nov. 1909 (BM); King George Sound, *R. Brown*, also *Mueller* (Oldfield?) (BM), also *Cunningham*, 1821?, and *Cunningham* 290/4th Voyage, 1821 (KEW) (photographs Nos. 1583, 1584 at CANB); Mt. Melville, King George Sound, *Oldfield* (KEW, PERTH); Mt. Melville, Albany, *C. Andrews*, 17.xii.1902, and Albany, *R. Helms*, Dec. 1898 (PERTH); "Swan River," *Drummond*, 112, and Coll. IV, No. 221 (KEW).

3. *Helichrysum obovatum* DC., *Prod.* 6: 180 (1838); Benth., *Fl. Austral.* 3: 627 (1867).

Holotype.—"Ad ripas Flum. Hastings ad Portum Macquarie legit cl. *Cunningham*" (G-DC) (non vidi) (isotype at Kew?).

Subshrub with weakly woody straggling stems. *Branchlets* woolly tomentose when young but later glabrescent. *Leaves* petiolate, obovate, 2–3 cm long, 1–1.5 cm wide, thin, glabrous above, with sparse woolly hairs or glabrescent below, apices rounded-obtuse, apiculate. *Panicles* terminal, branches woolly-tomentose, minute bracts more or less deciduous. *Involucres* turbinate-campanulate, 3–4 mm long. *Outer bracts* woolly, the innermost with small scarious laminae, all later reflexed. *Florets* 10–20(?). *Achenes* glabrous. *Receptacle* pitted.

This species differs from the two Western Australian ones in the lack of radiating laminae on the innermost bracts but short glabrous tips are usually present.

Specimens Studied.—NEW SOUTH WALES: Rocky slopes of the Hastings R., Port Macquarie, *A. Cunningham* 16, May 1819 (KEW); also *Cunningham*, 2nd Voyage Mermaid No. 43, 1818 (BM); N.S. Wales, *Fraser* 334, 1818 (BM).

4. *Helichrysum whitei* sp. et stat. nov.

H. obovatum var. *longifolium* White & Francis in *Bot. Bull.*, Brisbane 22: 28 (1920).

Holotype.—Upper Tallebudgera Creek, slopes of Macpherson Range, *J. E. Young* and *C. T. White*, December 1917 (BRIS, KEW).



Fig. 2.—A–A₄, *Helichrysum whitei* sp. nov. (drawn from Blake 14930). B–B₄, *H. bidwillii* Benth. (drawn from "Macleay R., Beckler" = syntype). Lettering as in Fig. 1 except that detailed sketches are all $\times 10$.

Weakly woody subshrub with slightly striate branches which are densely arachnoid-tomentose when young but later glabrescent. *Leaves* 5–9 cm long, 6–12 mm wide, linear-lanceolate-elliptical, narrowed below into a short petiole, triplinerved owing to the development of one pair of laterals, the nerves slightly prominent below. Upper surface glabrous (or rarely scabrid?) drying olive green, lower densely arachnoid-tomentose like the stems and inflorescence. *Panicles* spreading corymbose, the branches subtended by reduced leaf-like linear bracts. *Involucres* pedunculate, loosely clustered, 3–3.5 mm long; *outer bracts* densely tomentose, inner similar but with minute glabrous scarious tips; all slightly incurved at first but later reflexed. *Florets* 20–25. *Achenes* minutely pubescent, sometimes almost glabrous below, the pappus barbellate and slightly thickened above. *Receptacle* scarred by minute pits or merely rough. (Fig. 2, *A–A*₄.)

The more robust habit, thicker leaves of a very different shape, the denser vestiture, and the more densely tomentose and spreading panicle give this a very different appearance from *H. obovatum*. In the description of the latter Decandolle states that there are about 10 florets but Bentham, who quotes Beckler's Clarence River specimen mentioned below, says "about 20" and this specimen agrees closely with the holotype of *H. whitei*. There is also a further specimen from the same area collected by Binns and both of them have varietal names attached by Mueller. Apparently these varieties, based one on the white under-surface and the other on the marked scabridity of the leaves, were never published.

Specimens Studied.—QUEENSLAND: Lamington National Park at head of Upper Ballajui Falls, *S. T. Blake* 4930, "straggling perennial with stems ca. 2 ft.", 17.iv.1943; Lamington National Park, *C. T. White* 12547, 25.iv.1944 (BRI). NEW SOUTH WALES: Clarence R., *Beckler*; dp., *Binns* (KEW); Hastings R., *Betche* (BM).

5. *Helichrysum eriocephalum* J. H. Willis in Proc. Roy. Soc. Qd. (for 1950) 62: 101 (1952).

Holotype.—South Kennedy District, Lake Elphinstone, about 100 miles WSW. of Mackay, *Amalie Dietrich* 1722, 1870 (MEL) (clastotype BRI, isotype in BM).

Apparently a weakly woody plant as in other species of the section *Hebelaena* but the specimens too short to reveal whether there is a similar interrupted growth habit. *Branches* white-tomentose. *Leaves* lanceolate to elliptical-lanceolate, acuminate, both surfaces arachnoid-tomentose, the nerves prominent below. *Capitula* corymbosely clustered at the ends of the leafy branches, subhemispherical. *Florets* about 40. *Involucral bracts* numerous, the outer very webby-tomentose, the inner with spreading crumpled white laminae to 1 mm long. *Achenes* glabrous.

No over-mature capitula being available it is unknown whether the bracts are reflexed at a late stage but otherwise the affinity seems unquestionable. The species is known only from the type collection and differs from *H. whitei* in the smaller inflorescence, the larger number of florets, and the leaves with both surfaces tomentose.

6. *Helichrysum bidwillii* Benth., Fl. Austral. 3: 627 (1867).

Lectotype.—Wide Bay, *Bidwill* (KEW).

Syntype.—Macleay River, *Beckler* (MEL).

Weakly woody plants with a branching habit showing primary, secondary, and higher order series of flowering shoots. *Branchlets* thinly cottony woolly when young (more so in Bidwill than Beckler specimens) more or less glabrous later. *Leaves* narrowing into slender petioles, ovate, obovate or broadly elliptical, 1.5–3 cm long, 1–1.5 cm wide, acute to obtuse, commonly minutely apiculate, subglabrous above, webby below. *Capitula* in small loose clusters at the ends of the leafy laterals, subsessile, about 3 mm long, pale or straw-coloured. *Outer bracts* webby hairy, inner with minute white acuminate but not radiating laminae which may become torn, all bracts reflexed when over-mature. *Florets* about 20. *Achenes* glabrous (Bentham) or with few minute papillae when young (Beckler specimen). (Fig. 2, B–B₄.)

Though, with *H. vagans*, intermediate in character between the two sections this species has the growth habit, pithy stems, and reflexed persistent involucre bracts of the *Hebelaena* group.

Specimens Studied.—Apart from the Kew and Melbourne sheets there is one from Killarney, collected J. L. Boorman, iii.1911, at the British Museum. This locality is near the Queensland–New South Wales border to the south-east of Warwick.

7. *Helichrysum vagans* C. T. White in Proc. Roy. Soc. Qd. (for 1938) 50: 80 (1939).

Holotype.—Lamington National Park, McPherson Range, alt. 1000 m, C. T. White, Feb. 1920 (KEW, BRI).

Straggling shrub to 2 m high, with angular branchlets that are thinly tomentose when young but later glabrescent. *Leaves* linear-lanceolate to lanceolate, 2.5–4 cm long and 4–5 mm wide on flowering shoots but up to 10 cm long and 15 mm wide on vegetative ones, trinervate. *Capitula* in subglobose clusters terminal to the upper laterals or in a terminal corymb, campanulate, 3 mm long, 3 mm in diameter. *Involucre bracts* scarious, brownish when dry; outer sparsely woolly or glabrous; inner stiff above but with very narrow membranous margins, the tips not radiating. *Florets* about 12. *Achenes* glabrous.

In his description White stresses a relationship with *H. beckleri* F. Muell. ex Benth., but examination suggests that there is a closer affinity to *H. bidwillii*. There is a similar growth habit though the angular and more glabrous stems, the involucre bracts without white tips, and the general robustness serve to distinguish it. It is near to *Ozothamnus* sens. str., the bracts being less definitely reflexed after flowering, but the stems are less woody and the general appearance and character place it with species in the section *Hebelaena*. White states that the species is associated with the edges of rain-forest clearings in mountain localities.

Specimens Studied.—QUEENSLAND: Echo Point, Lamington National Park, "shrub 1.5 m of rather straggling growth, common on cliff edges bordering rainforest", C. T. White 12039, 19.ii.1943; Springbrook, "shrub 6–7 feet high, scrambling habit, growing in rainforest", C. T. White, January 1916; do., "shrub of straggling growth 2 m. high, common on edge of rainforest. Flower heads white", C. T. White 8226, 25.x.1931. NEW SOUTH WALES: Mt. Warning, alt. 1000 m, C. T. White and L. J. Brass 354, Jan. 1938 (BRI).

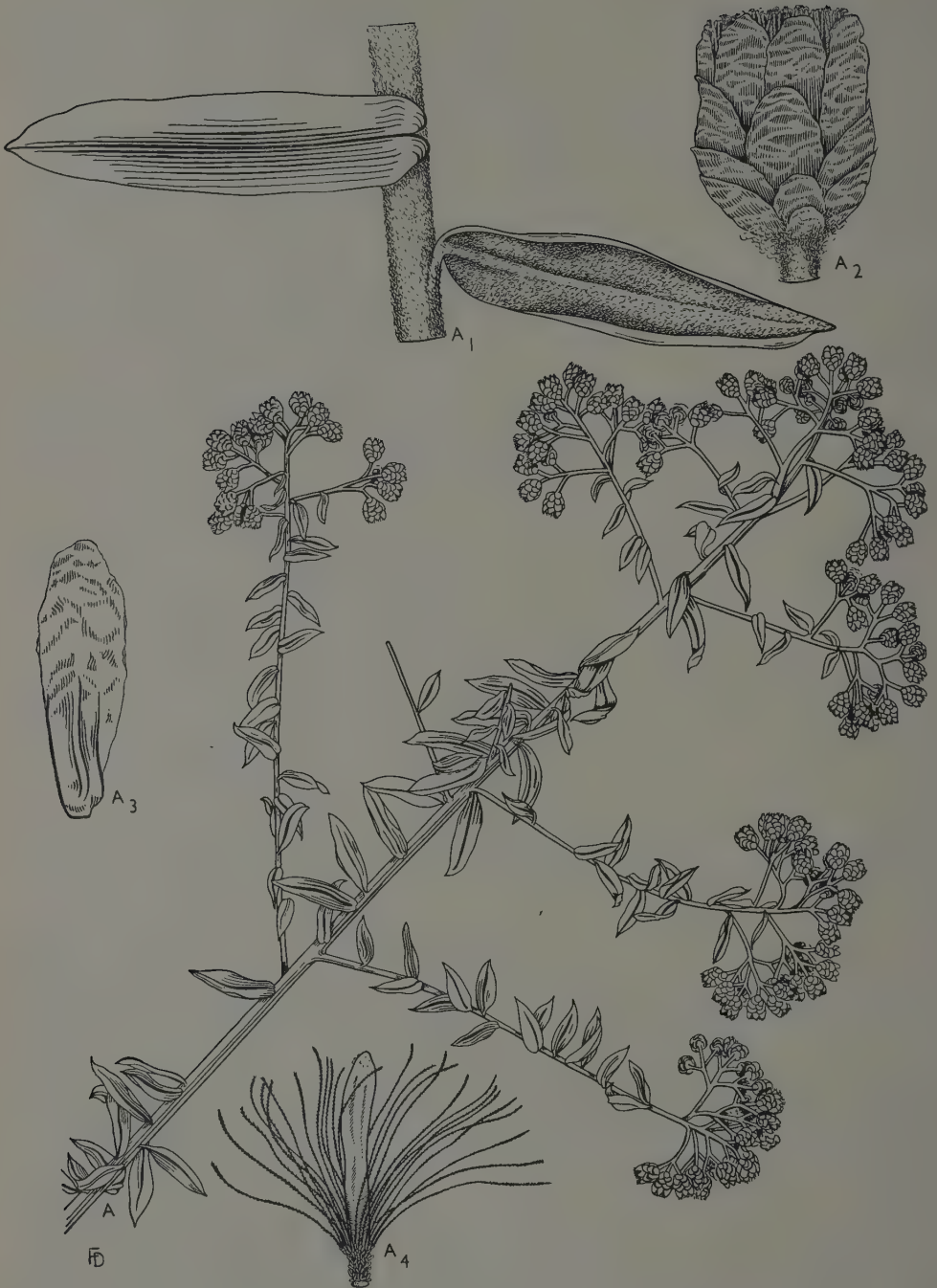


Fig. 3.—A–A₄, *H. rufescens* (DC.) comb. nov. (drawn from Gray 3012). Lettering as in Fig. 2.

8. *Helichrysum rufescens* (DC.) comb. nov.

Ozothamnus rufescens DC., Prod. 6: 165 (1838); *Ozothamnus beckleri* F. Muell., Fragm. 1: 183 (1859); *Helichrysum beckleri* F. Muell. ex Benth., Fl. Austral. 3: 627 (1867).

Holotype.—Port Jackson, *Gaudichaud-Beaupre* (G-DC).

Holotype of Synonym.—Hastings R., *Beckler* (MEL, KEW).

Erect much branched shrub, the branches tomentose when young but later glabrescent. *Leaves* very shortly petiolate, narrow-lanceolate to oblong-lanceolate, apiculate, 1–2 cm long, 3–5 mm wide, glabrous above, tomentose below. *Capitula* terminal in spreading floriferous pyramidal panicles of which the branches are subtended by minute leafy bracts. *Involucres* 3–4 mm long, campanulate; *outer bracts* scarious, rufescent or pale; *inner* with stiff narrow claws and oblong thinly scarious limbs, all horizontally spread after the shedding of the achenes but not deciduous with them and apparently not or rarely reflexed. *Florets* about 20. *Achenes* papillose-pubescent (immature). (Fig. 3.)

Bentham makes no reference to *O. rufescens* in the “Flora Australiensis”, nor has any mention of it been found in the literature. Nevertheless a comparison of the descriptions and of a fragment and a photograph of the type at Geneva with the Melbourne material shows that *H. beckleri* is undoubtedly conspecific with it.

Specimens Studied.—(Note.—Owing to an oversight the material at Kew was not listed but it was taken into account at the time the above description was prepared.) QUEENSLAND: Springbrook, alt. 1000 m, large dense growing shrub 10 ft high, very common as second growth. Leaves dull dark green above, white beneath. Flowers white—*C. T. White* 6348, 20.ix.1929; Springbrook, McPherson Range, margin of a dense rain-forest, alt. 3000 ft, *C. E. Hubbard* 4189, 28.ix.1930. NEW SOUTH WALES: Dorrigo Mt., *M. Gray* 3012, 17.ix.1954; Dorrigo, *J. L. Boorman*, ix.1909; “Native of the Devils Glen in the Interior,” N.S. Wales, *Fraser*, 1818; off Doyle’s River Road, about 48 miles W. of Wauchope, *N. C. Ford*, 6.ix.1951 (BM).

9. *Helichrysum cassinioides* Benth., Fl. Austral. 3: 630 (1867).

Lectotype.—Keppel Bay, *R. Brown* (Bennett No. 2203) (KEW).

Erect shrub with tomentose branches which later become webby or glabrescent. *Leaves* linear, 1.5–3 cm long, acute or obtuse apiculate, channelled above with a little wool along the midrib, revolute along the margins and densely tomentose below. *Capitula* in corymbose or pyramidal clusters terminating many of the upper branchlets. *Involucres* cylindrical to narrow turbinate, pale brown, 5–6 mm long; *outer bracts* ovate, horny-chartaceous, slightly striate, *inner* thinner, paler and with thin margins, the laminae oblong and about twice as long as the thick claws which bear “globule” hairs on the dorsal surfaces. *Florets* 12–15. *Achenes* glabrous or minutely hairy, pappus bristles scarcely thickened above.

The sheet at Kew bears three pieces. Associated with one is a small label, in Robert Brown’s hand, giving a manuscript name under *Ozothamnus* and “Keppel Bay”. From the script this was probably written some years after collecting and certainly later than 1817. A piece in the lower left-hand corner of the sheet bears “East Coast” in Brown’s earlier script. The third piece has no special label but

appears to match the latter. There is also one of the labels distributed with the duplicates when they were sorted out by J. J. Bennett. This gives "No. 2203 *H. cassinioides* Bth. Keppel Bay Broad Sound" and, apart from the number, was written by Bentham. Brown's specimens were not distributed from the British Museum until some years after the publication of volume 3 of the "Flora Australiensis", but there is no indication as to when Bentham prepared this label. Nevertheless examination of the sheet at the British Museum shows that Brown's material was collected at Keppel Bay on August 15, 1802. According to Brown's diary for this date he went ashore and "landed on the main at the bottom of a little hill near the entrance of one of the arms of the bay. This hill is inundated at high water. We found on it a few plants we had not hitherto met with . . . in the evening went on shore again and had a short interview with the natives who had come to the beach".

There is also a sheet at Melbourne labelled "*H. cassinioides* Bth. East Coast" in another hand and this agrees with the East Coast fragments at Kew. There is no doubt of its conspecificity.

10. ***Helichrysum diosmifolium*** (Vent.) Sweet, Hort. Brit., 1st Ed., 223 (1826); Less. in Steud., Nom. Bot., 2nd Ed. (1840); *Ozothamnus diosmaefolius* DC., Prod. 6: 166 (1838); *Gnaphalium diosmaefolium* Vent., Jard. Malm. 74 (1804).

Holotype.—Redoute's figure in the "Jardin Malmaison" (see note below).

Erect shrub to 3 m high, branches woolly-scabrid when young, later spreading-scabrid or glabrescent. *Leaves* crowded, linear, closely revolute below (rarely elliptical when not revolute), 1–2 cm long, 0.5 mm in diameter, with a minute reflexed apiculum, scabrid or almost smooth. *Capitula* numerous in terminal and almost leafless corymbose panicles, subglobose and with 20–30 florets. *Involucral bracts* milky white, opaque and stiff, obtuse, concave and slightly incurved; the *outermost* often pink-flushed when young; the *inner* with stiff narrow claws and opaque laminae; all spreading when over-mature but not reflexed and apparently not deciduous with the papillose achenes.

The species occurs from the coast to the western slopes in New South Wales and extends into south-eastern Queensland (vide Willis in Proc. Roy. Soc. Qd. 62: 104 (1952)).

According to information received from Geneva the only specimen of special significance is one labelled "Tic d'Ventenat, h Malm. 23 7b 1816.". Reference to Paris failed to locate any other possible type. It is possible that "Tic" should be read as "recu." but the date, which may be that of receipt, is so late that the specimen cannot be proved to be the type.

Ventenat's publication not being available to the author, the text was checked at Kew by Dr. R. Melville, who writes "no specimens are cited. He says 'originaire de Cap de Bonne-Esperance' a misconception that Decandolle later corrected. Ventenat's work describes plants in cultivation and the fact that he did not know the true country of origin of this one proves that he did not have any native material

before him". From this it follows that the species must be regarded as based on the figure by Redoute in the "Jardin Malmaison".

11. *Helichrysum kempei* F. Muell. in Chem. & Drugg. (with Austral. suppl.) No. 45: 67 (June 1882).

Lectotype.—Finke R., *Kempe*, 393, in 1880.

Shrub to 1 m high with spreading tomentose branches and the habit of *Cassinia*. *Leaves* linear, papillose-scabrid above the lower tomentose surface commonly obscured by the closely revolute margins, 2–3 cm long, minutely apiculate. *Capitula* in corymbose leafless panicles not much longer than the upper leaves, turbinate-campanulate, 5 mm long, 2.5–3 mm in diameter. *Florets* 10–15. *Involucral bracts* thinly scarious, obtuse, pale, the outer glabrous or with few woolly hairs, the inner with centrally thickened claws bearing coiled woolly hairs and swollen papillae which look like globules of golden brown exudate and with scarious laminae which are slightly incurved or erect. *Bracts* deciduous with or after the achenes. *Achenes* papillose, pappus bristles thickened above.

There are three *Kempe* specimens, all from Finke River in central Australia, in the Melbourne Herbarium. As well as No. 393 there are Nos. 21 and 305, but all are small and somewhat fragmentary. The species has also been collected in Standley Chasm, W. of Alice Springs (Burbidge, 4157, 18.xi.1955), where it occurred in rocky crevices of the steep cliffs. The isolated occurrence so far from closely related species emphasizes the refugial nature of some of the flora of the MacDonnell Ranges.

12. *Helichrysum tuckeri* F. Muell. ex J. H. Willis in Proc. Roy. Soc. Qd. (for 1950) 62: 102 (1952).

Holotype.—Lachlan R., *Gerard Tucker*, 1879 (MEL).

Rigid shrub to 1.25 m high, branches thinly tomentose. *Leaves* appressed, 3–4 mm long, glabrous, narrow-oblong, slightly swollen at the base but not auriculate as in *H. diotophyllum*, closely revolute above, obtuse. *Capitula* shortly pedunculate in small terminal corymbs, campanulate, 3–4 mm in diameter, 7–12-flowered. *Involucral bracts* glabrous or with few woolly hairs below; *outer* orbicular; *inner* with obovate-oblong white laminae and narrower stiff claws. *Achenes* densely scabrid-papillose, the pappus bristles thickened above.

As Willis shows, this is a species of the western slopes in New South Wales. The involucre has some resemblance to those in *H. diosmifolium* but they are much less numerous. The leaf characters suggest affinities with both *H. adnatum* and *H. diotophyllum*.

13. *Helichrysum diotophyllum* F. Muell., Fragm. 5: 150 (1866).

Holotype.—Dogwood Creek, *Leichhardt and Bunce* (MEL, KEW).

Rigid, much branched shrub to 1.5 m high, branches tomentose when young, later glabrescent. *Leaves* 3–5 mm long, appressed, glabrous except for a thin line of tomentum visible between the closely revolute margins below, linear except for

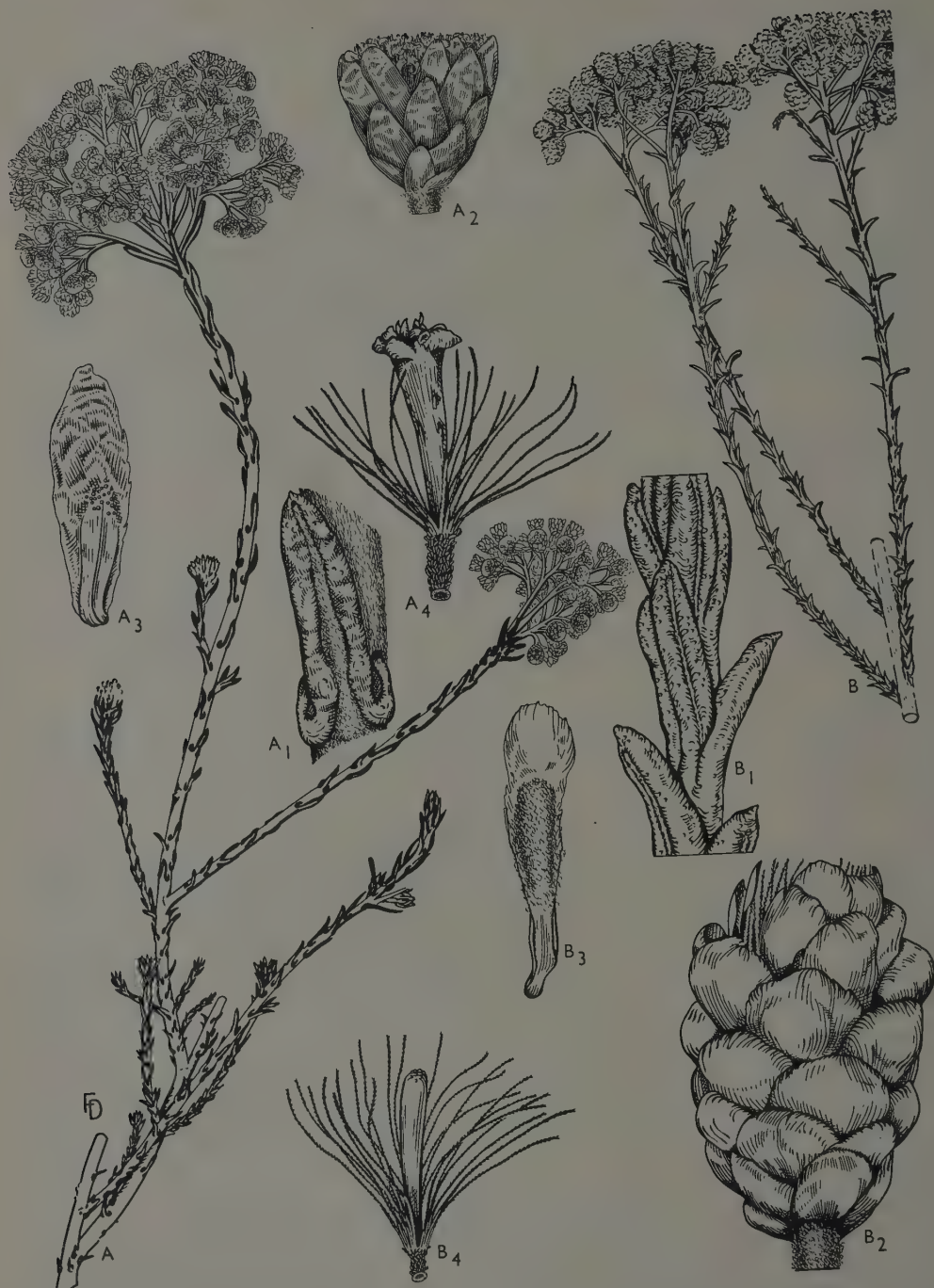


Fig. 4.—A–A₄, *H. diotophyllum* F. Muell. (drawn from Everist 3507). B–B₄, *H. adnatum* (DC.) Benth. Lettering as in Fig. 2.

the curious stem-clasping auricles, and with a minute oblique apiculum. *Capitula* in dense corymbose panicles at the ends of elongated shoots, shortly pedunculate, bracteate. *Involucres* 3–3.5 mm long, campanulate, c. 30-flowered; *outer bracts* ovate, obtuse, almost or quite glabrous; *inner* with oblong obtuse laminae and thickened claws; all scarious and pale. *Achenes* papillose, pappus bristles not particularly thickened at the tips. (Fig. 4, A–A₄.)

The peculiar character of the leaves of this species is best seen along the elongated shoots. On vegetative shoots the leaves are more crowded. The innermost bracts may bear a few woolly hairs and also some of the “globule” papillate hairs found in various species.

The distribution has been discussed by Willis (in Proc. Roy. Soc. Qd. (for 1950) 62: 104 (1952)), where it is shown to extend from the Taroom area in Queensland to the more western parts of the western slopes in New South Wales.

14. *Helichrysum cassiope* Sp. Moore in J. Linn. Soc. 34: 199 (1899).

Holotype.—Gibraltar, W.A., Spencer Moore (BM).

Shrub 1–1.5 m high, the branchlets lanose-tomentose or closely webby when young but later partially glabrescent. *Leaves* 2.5–3 mm long, narrow linear, obtuse, scabrid-wrinkled and sparsely hairy above, revolute and puberulous below, crowded and appressed with narrow widely divaricate stem clasping auricles which are obscured by the vestiture when young. *Capitula* in small terminal subglobose loose corymbs. *Involucres* narrow-turbinate (at least when young), 3–4 mm long, bracts pale straw-coloured, concave, scarious; *outer bracts* obovate, obtuse; *inner* with oblong laminae and narrow claws. Florets about 12–16. *Achenes* glabrous (Sp. Moore) or minutely papillose (Royce, No. 5337), pappus bristles slightly thickened above. (Fig. 5, B–B₄.)

This species is restricted to Western Australia. It is known from the original site, which is to the SW. of Coolgardie, and from a specimen collected “3 miles N. of Cundeelee Mission, N. of Zanthus, R. D. Royce 5337, 27.i.1956” (PERTH, CANB). Zanthus is about 120 miles E. of Kalgoorlie, so that the two localities are in the same ecological belt. While Royce 5337 is believed to represent the taxon described by Spencer Moore it differs from his description in that the bases of the leaves are markedly divaricate and stem-clasping (less so on very young stems) and the achenes are papillose. However, a report recently received from the British Museum states that this character is also shown in the original material.

15. *Helichrysum tessellatum* J. H. Maiden & R. T. Baker in Proc. Linn. Soc. N.S.W. (2)10: 589, t. 53 (1896); *H. brevidecurrens* J. H. Maiden & R. T. Baker, loc. cit.

Holotype.—Byalong, Murrumbo (Goulburn R.), N.S.W. (KEW, SYD).

Holotype of Synonym.—Murrumbo, Goulburn R., N.S.W. (KEW, SYD).

Erect shrub with tomentose branches on which the decurrent bases of the leaves are persistent. *Leaves* linear, 1.25–2 cm long, revolute, tomentose below, adherent to the stem so that up to a third of the length is decurrent, apices apiculate.

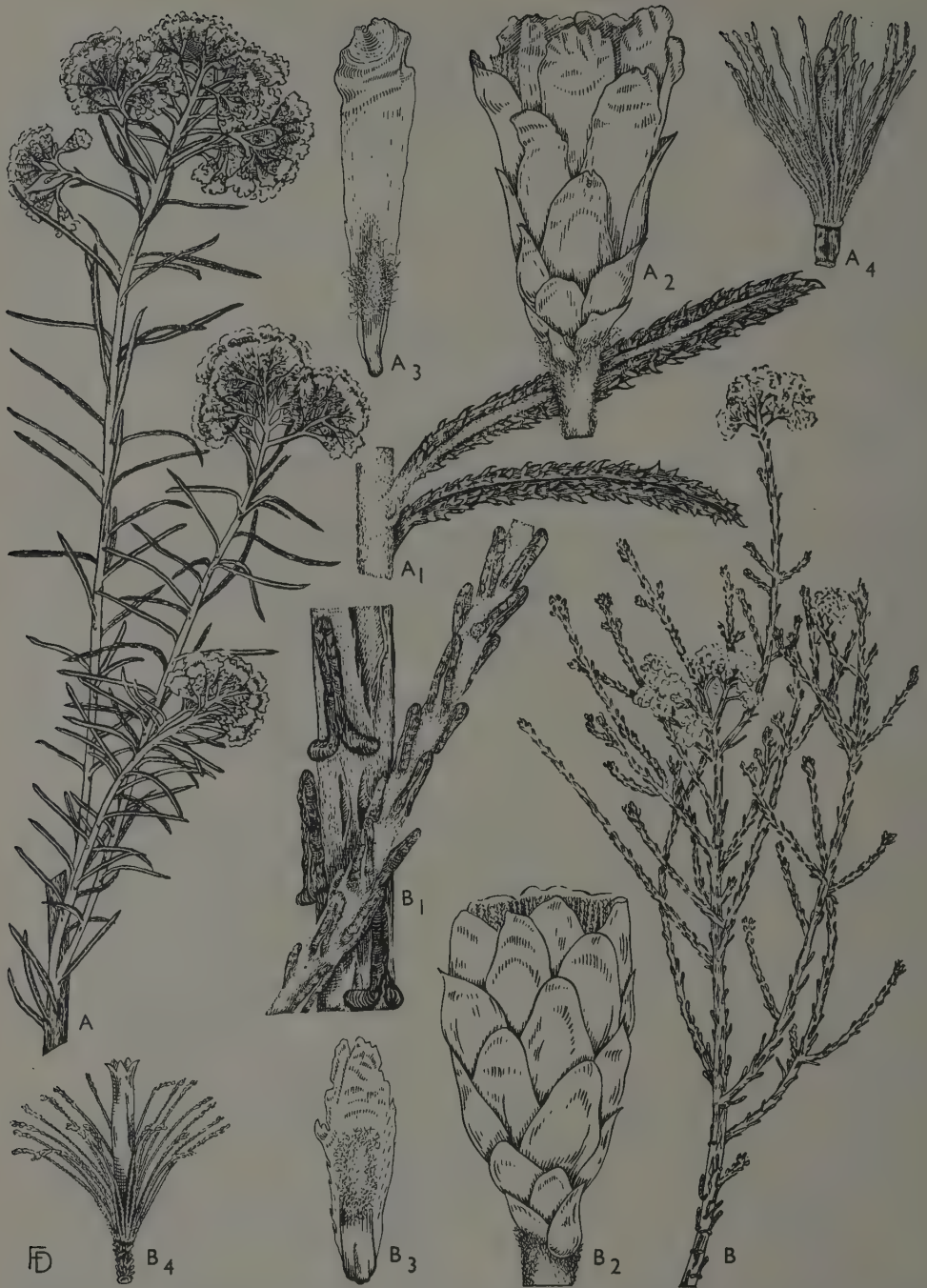


Fig. 5.—A-A₄, *H. rosmarinifolium* (Labill.) Steud. ex Benth. (drawn from "Collinsville, C. E. Lord"). B-B₄, *H. cassiope* Sp. Moore (drawn from Royce 5337). Lettering as in Fig. 2.

Capitula densely corymbose, the peduncles bracteate, turbinate-campanulate, 4–7 mm in diameter. *Involucral bracts* scarious and straw-coloured, *outer* orbicular with few hairs at the base, *inner* with ovate laminae and short claws bearing woolly hairs. *Florets* 30–40. *Achenes* hairy, the pappus bristles not thickened upwards.

There can be no doubt that Willis (Proc. Roy. Soc. Qd. (for 1950) 62: 103 (1952)) is correct in stressing the close relationship of this species with *H. adnatum* and with *H. brevidecurrens*. The comparison which Maiden and Baker made with *H. bracteolatum* is misleading and it must be doubted whether they had seen an authentic specimen of Hooker's species at this date.

The only specimens studied have been the duplicates of the types sent to Kew by Maiden.

16. *Helichrysum adnatum* (DC.) Benth., Fl. Austral. 3: 628 (1867) quoad syn. excl. spec. cit.; *Ozothamnus adnatus* DC., Prod. 6: 166 (1838).

Holotype.—Port Jackson, Gaudichaud-Beaupre (G-DC).

Shrub with woolly tomentose branches on which the adherent leaf bases may persist. *Leaves* linear-oblong, obliquely apiculate, 3–8 mm long, glabrous or scabrid above, tomentose below between the revolute margins, adherent to the stem for up to half their length and slightly spreading at the point of attachment. *Capitula* globular when young, in corymbose panicles terminal to the branches, 3–4 mm long. *Involucral bracts* opaque, whitish or yellow, the *outer* ones orbicular with rounded apices, *inner* with ovate obtuse or rounded laminae and broad claws whose thickened centres bear short woolly hairs as well as swollen "globule" ones. The apices of the bracts often split when old. *Florets* 12–22. *Achenes* hairy, the pappus not much thickened towards the ends. (Fig. 4, B–B₄.)

It is unfortunate that under this combination, which must stand according to Article 65(2) of the International Code, Bentham based his description on specimens which do not agree with the type used by Decandolle of which a photograph and a fragment have been examined. The confusion which has resulted has been discussed by various authors, including Maiden in his "Illustrations of New South Wales Plants", part i, p. 20 (1907). Maiden's differentiation between this species and *H. diotophyllum* is correct but since none of the specimens quoted from the "Flora Australiensis" is true *H. adnatum*, his notes on distribution cannot be accepted.

The inflorescences are larger than in *H. diotophyllum* and also more corymbose. The method of decurrence is not the same as that exhibited in *H. bilobum* and *H. catadromum*, where there are lines of tissue running from the point of attachment to the node below.

The distribution has been discussed by Willis (Proc. Roy. Soc. Qd. (for 1950) 62: 103–4 (1952)). The species occurs in the central tablelands and the central and southern coastal regions of New South Wales and has also been reported from Suggan Buggan in north-eastern Victoria.

17. ***Helichrysum blackallii* sp. nov.**

H. adnatum sensu J. H. Maiden, Illustrations of N.S.W. Plants, pt. i: 20 (1907) pro parte.

Holotype.—Kumarl, L. A. Hornbury 135, Sept. 1938 (Blackall Herbarium—PERTH).

Affinis *H. adnato* sed foliis adhaerentibus et decurrentibus, capitulis oblongis, flosculis paucioribus differens.

Frutex, rami glabrescentes sed primo tomentosi. *Folia* linearia, prope basin adhaerentia et decurrentia, supra scabridula, subter tomentosa, marginibus revoluta. *Corymbae* terminales. *Capitula* breviter pedunculata, oblonga. *Squamae* extimae concavae, chartaceae, stramineae vel lacteolae. *Laminae squamarum intimarum* simulantes sed lacteolae, bases angustae, sparsim villosae. *Florulae* 12–14. *Achaenia* papilloso-pubescentes, pappi setae vix crassatae in apice.

Small shrub whose glabrescent branches are tomentose on the younger parts and on the inflorescence. *Leaves* intermediate in character between those of *H. adnatum* and *H. catadromum*, linear, revolute on the margins and adherent to the stem for more than half their lengths, the lines of decurrent tissue extending almost to the leaf immediately below, 4.5–8 mm long, minutely scabrid above, tomentose below between the margins. *Capitula* pedunculate, in terminal clusters or corymbs, distinctly oblong in outline though loosely spreading above when older, 5–6 mm long. *Involucral bracts* concave and incurved, outermost pale, the remainder with milky-white laminae and claws with few woolly hairs and numerous “globules”. *Florets* 12–14. *Achenes* papillose-hairy, the pappus bristles scarcely thickened above.

Maiden included a specimen of this under his discussion of *H. adnatum*, but apart from the completely discontinuous distribution this species can be recognized by the greater degree of adherence of the leaves to the stem, the oblong capitula, and fewer florets. At first glance it bears a distinct resemblance to *H. catadromum*, from which it differs in the milky white bracts and their lack of membranous margins. As in other concave bracted species the margins are apt to split even in unexpanded involucre.

Specimens Studied.—WESTERN AUSTRALIA: Salmon Gums, G. H. Burvill, November 1932; sine loc. W. E. Blackall 7 (PERTH). SOUTH AUSTRALIA: Fowler's Bay, A. Richardt (SYD).

18. ***Helichrysum bilobum* subsp. *bilobum*.**

H. bilobum N. A. Wakefield in Vict. Nat. 68: 51 (1951); *Ozothamnus retusus* Sond. & Muell. in Linnaea 25: 510 (1852); *Helichrysum retusum* (Sond. & Muell.) F. Muell., Fragm. 8: 46 (1873) non Spreng.; *H. adnatum* Benth., Fl. Austral. 3: 629 (1867) pro parte.

Lectotype.—“On the summits of pine hills at the right side of Gawler”—Behr (MEL).

Much branched shrub thinly tomentose between the angularities of the decurrent tissue on the stems. *Leaves* linear, revolute on the margins but less so at the apices where the recurved tips may give a bilobed appearance, 7–25 mm long,

markedly decurrent, the lines of tissue extending to the leaf immediately below, glabrous or minutely scabrid, with a slight but sometimes rather inconspicuous constriction at the point of junction with the stem. *Capitula* numerous in corymbose panicles terminal to the branches, cylindroid or cylindrical-turbinate, 3–4 mm long. *Involucral bracts* thinly tomentose below and stiffly opaque but pale above, the inner with oblong laminae which are centrally opaque and a thickened claw bearing few woolly hairs and numerous “globules”. *Florets* 10–12. *Achenes* glabrous, pappus bristles slightly thickened above.

The specimens show certain variations which can be sorted loosely into Victorian and Kangaroo Island (with some South Australian mainland) plants. In the former, and also in the lectotype, the leaves are all much the same length and of a colour similar to that of the green stem, but in the latter group there are longer leaves with short axillary shoots bearing short leaves. The stems in these are brownish and the leaves conspicuously darker above. Whether special consideration should be given to such variations is not, at present, clear since the taxa as known are not sufficiently distinct one from the other.

The description given by Bentham, for *H. adnatum*, is evidently based on material of this species and, with the exception of the Dallachy and Goodwin collection from the Darling River, all the specimens quoted by him in the “Flora Australiensis” under this name belong with *H. bilobum*.

The distribution is through western Victoria, south-western New South Wales and southern South Australia.

18a. *Helichrysum bilobum* subsp. *scabrum* comb. nov.

Ozothamnus scaber F. Muell. in *Linnaea* **25**: 407 (1852); *Helichrysum adnatum* var. *scabrum* (F. Muell.) Benth., *Fl. Austral.* **3**: 629 (1867).

Lectotype.—Cudnaka, *F. Mueller* (MEL).

Differs from the above chiefly in the coarse scabridity of the often larger leaves (to 2 cm long) with more closely revolute margins and without a flat portion before the retuse apices. The inflorescences and capitula are very similar to those of the above.

This subspecies is represented by a Mueller specimen at Kew and Melbourne and by one at the British Museum labelled “Mt. Arden Ranges, *T. S. Lea*, March 25, 1885–6”.

19. *Helichrysum catadromum* N. A. Wakefield in *Vict. Nat.* **68: 51 (1951);**

Ozothamnus decurrens F. Muell. in *Trans. Phil. Inst. Vict.* **3**: 59 (1857);

Helichrysum decurrens F. Muell., *Fragm.* **8**: 46 (1873) non Moench.

Lectotype.—Lower Murray Desert, *F. Mueller* (MEL).

Much branched shrub with angular branches due to the persistent decurrent bases of the leaves. *Leaves* linear, 3–6 mm long, channelled above and so wrinkled as to seem almost bullate, revolute, slightly retuse, obliquely apiculate, constricted at point of attachment, the lines of decurrent tissue so close as to almost obscure the tomentum. *Capitula* shortly pedunculate, clustered in small corymbs, narrow-

turbinate, c. 5 mm long. *Involucral bracts* straw-coloured, chartaceous rather than scarious, obtuse, thinly membranous on the margins; *outer* woolly on the back below the middle; *inner* with stiff narrow claws bearing woolly hairs and numerous "globules". *Florets* 10–12. *Achenes* papillose-scabrid, pappus bristles not particularly thickened above. (Fig. 6, B–B₄.)

The thin ragged margins of the involucral bracts, the narrow involucre, and the leaf character serve, in combination, to distinguish this from *H. bilobum*, *H. blackallii*, and *H. occidentale*. It is represented at Kew by the Dallachy and Goodwin specimen referred to *H. adnatum* by Benth. in the "Flora Australiensis". It is found in the drier areas of southern Australia (Wakefield, loc. cit.) but seems restricted to the eastern regions.

20. *Helichrysum occidentale* sp. nov.

H. adnatum et *H. decurrens* sensu C. A. Gardner, Enum. Pl. Austral. occ. 134 (1930).

Holotype.—Kumarl, "compact shrub $\frac{1}{2}$ –2 feet", L. A. Hornbury, 145, Sept. 1938 (Blackall Herbarium, Perth Museum, W.A.).

Affinis *H. catadromo* sed foliis scabridis, bracteis induratis albicoloris concavis differens.

Frutex. *Folia* *H. catadromo* simulans sed scabridis. *Corymbae* terminales. *Capitula* breviter pedunculata, obovoidea, 3.5–4 mm longa. *Squamae* induratae, concavae, lacteolae. *Florulae* 6–8. *Achaenia* papillosa.

The general habit approaches that of *H. catadromum* closely and treatment as a subspecies was considered. The differences are: *Leaves* scabrid and not minutely bullate when dry, the decurrent tissue of the stem similar. *Capitula* in small terminal clusters, 3.5–4 mm long, obovoid owing to the concave milky white bracts. *Inner involucral bracts* with chartaceous white laminae and stiff narrow brownish claws whose thin vestiture does not conceal the numerous "globules". *Florets* 6–8. *Achenes* minutely papillose and angular when young, the pappus bristles slightly thickened above.

The scabridity of the leaves suggests an affinity with *H. bilobum* subsp. *scabrum* but the involucre, despite the smaller size and fewer florets, resemble those of *H. blackallii*. It is restricted to the southern Eremaea in Western Australia.

Specimens Studied.—WESTERN AUSTRALIA: Near Southern Cross, "shrub 2–3 feet", W. E. Blackall 870, Oct. 1930 (PERTH and Herb. Blackall); Mt. Holland, C. A. Gardner, July 1929 (PERTH).

21. *Helichrysum obcordatum* subsp. *obcordatum*

Helichrysum obcordatum (DC.) F. Muell. ex Benth., Fl. Austral. 3: 632 (1867); *Ozothamnus obcordatus* DC., Prod. 6: 165 (1838).

Holotype.—Insula Van Diemen, Lesson, Exped. de l'Astrolabe (G-DC).

Shrub to 1.5 m high with slightly angular branches. *Leaves* obovate to obcordate, 3–5 mm long, the apiculate tips of the midrib reflexed, narrowed below

into short slender petioles linked with slender lines of decurrent tissue which may be particularly obscured by the tomentum on the younger branchlets or where the internodes are short; upper surfaces green and glabrous, lower with a close flat grey or rusty tomentum in which the midrib is slightly raised; the margins slightly recurved and minutely irregular as if subscabrid. *Capitula* in spreading corymbose panicles, very numerous, cylindroid-turbinate, 2·5–4 mm long. *Involucral bracts* straw-coloured, shining, oblong, concave, obtuse but sometimes split; *inner* with scarious laminae and stiff claws which are almost glabrous except for numerous “globule” hairs. *Florets* 6–10. *Achenes* angular, papillose, pappus bristles slightly thickened upwards, about 12 in number. (Fig. 6, A–A₄.)

The photograph of the type sheet received from Geneva shows a label as follows: “Van Diemen M. 1829, exped. de l’Astrolabe /16”, there being a series of dots connecting the writing on the left of the label with that on the right.

This small-leaved variety is common on the hills near Hobart in Tasmania and extends from there through Victoria to the western slopes in New South Wales.

21a. *Helichrysum obcordatum* subsp. **major** stat. nov.

Helichrysum obcordatum var. *major* Benth., Fl. Austral. 3: 632 (1867);
Cassinia obovata DC., Prod. 6: 155 (1838).

Lectotype.—Twofold Bay, A. Cunningham (G-DC) (isotype KEW).

This taxon has larger leaves (7–18 mm long) that are cuneate to obovate, the apiculum is not so conspicuously reflexed, the margins are never recurved, and the tomentum is often visible extending minutely beyond them. The inflorescences are slightly larger and the branchlets more loosely tomentose. *Capitula* 5–6 mm long but otherwise closely similar to those of subsp. *obcordatum*.

The distribution is from the Grampians in Victoria to the Nandewar Range and to Wallangarra in north-eastern New South Wales. It seems to be generally associated with a higher altitude than is subsp. *obcordatum* on the mainland.

The Geneva specimen has not been seen and the above description is based on the specimens at Kew quoted by Benthham under his variety and on a suite of specimens in the Sydney Herbarium. The latter have been labelled by the author and may be consulted by those interested.

22. *Helichrysum lepidophyllum* (Steetz) F. Muell. ex Benth., Fl. Austral. 3: 633 (1867); non Tovey & Morris (1923); *Ozothamnus lepidophyllus* Steetz in Lehm., Pl. Preiss. 1: 468 (1845); *Helichrysum steetzianum* Tovey & Morris in Proc. Roy. Soc. Vict. (n.s.) 35: 196 (1923).

Syntype.—King George Sound, Oct. 1840, Preiss 28 (MEL).

Small shrub, thinly tomentose on the short laterals and the inflorescence, older portions glabrescent and with stringy or fibrous bark. *Leaves* minute, strongly reflexed, ovate to orbicular, obtuse, 0·5–1 mm long, glabrous above, tomentose below apart from the recurved margins, linked with decurrent lines of green tissue from node to node on the longer internodes but these obscure on the short laterals.



Fig. 6.—A–A₄, *H. obcordatum* subsp. *obcordatum* (drawn from “Bellerive, Atkinson”). B–B₄, *H. catadromum* N. A. Wakefield (drawn from holotype). Lettering as in Fig. 2.

Capitula in corymbose clusters terminating the upper branchlets, oblong-cylindrical, 3–5 mm long. *Outer involucrel bracts* concave-orbicular and pale-opaque; *inner* with milky white almost hooded laminae about half as long as the stiffer claws, which bear a few woolly hairs and numerous “globules”. *Florets* 6–9. *Achenes* papillose, the pappus bristles very slightly thickened above.

The capitula of this species suggests an affinity with *H. blackallii*, being more or less oblong in outline, but the minute reflexed and differently shaped leaves and the much less evident decurrent lines on the stems as well as the papillose rather than papillose-hairy achenes make the two quite distinct.

Apart from the material at Melbourne the type of this species is presumably in the Sonder Collection at the Naturhistoriska Riksmuseum in Stockholm. Its nomenclature has been discussed by Morris and Willis (Vict. Nat. 59: 85 (1942)) and further remarks may be found under *H. hookeri* below. It is restricted to low-rainfall areas of the South West Province of Western Australia.

Specimens Studied.—WESTERN AUSTRALIA: Dumbleyung, *F. M. C. Schoch* 128, 8.xii.1916; do., *C. A. Gardner*, December 1922; between Ellen's Peak and Tolls Creek, Stirling Range, *A. Morrison*, 23.x.1902; Albany, *W. E. Blackall*, December 1937; Middle Mt. Barren, *C. A. Gardner* 9164, 20.ix.1948; Mt. Ragged, *H. Steedman*, October 1931; E. of Ravensthorpe, *G. R. Meadly*, November 1933; sandplains near Ravensthorpe, *C. Andrews*, October 1903 (Herb. Blackall); near C. Arid, *Maxwell*; mallee country N. of Esperance, *W. E. Blackall* 25 (PERTH). (See also specimens quoted by Bentham in *Fl. Austral.*)

23. *Helichrysum reticulatum* (Labill.) Less. in Steud., *Nom. Bot.*, 2nd Ed. (1840); *Chrysocoma reticulata* Labill., *Pl. Nov. Holl.* 2: 40.t.183 (1806); *Faustula reticulata* Cass. in *Dict. des Sc. Nat.* 16: 252 (1820); *Gnaphalium reticulatum* Spreng., *Syst.* 3: 471 (1826); *Ozothamnus reticulatus* DC., *Prod.* 6: 164 (1838); Hook. f., *Fl. Tasm.* 1: 202 (1856).

Holotype.—“Capite van Diemen”, *Labillardiere* (FI?) (isotype KEW).

Shrub to 3 m high, tomentose on the branches, inflorescences, and under-surfaces of the leaves, stems stout. Leaves linear, 3–5 cm long, obtuse, revolute on the margins, with scattered glandular (globular-headed) hairs on the upper surface especially along the sunken midrib and commonly marked with transverse wrinkles at least when dry. *Capitula* in dense corymbose panicles terminal to the branches, shortly pedunculate and subtended by minute bracts, 6–7 mm long, campanulate. *Outer involucrel bracts* densely tomentose except on the scarious, acute or obtuse tips which commonly show (under $\times 40$) minute “globule” hairs, the margins minutely fimbriate or ragged; *inner* with acuminate or acuminate-acute whitish fimbriate laminae and membranously margined claws that are loosely woolly on the back. *Florets* at least 35. *Achenes* densely papillose-villous, the pappus sub-plumose.

The type locality of this species is obscure. The only Cape van Diemen on modern maps of Australia is a point on Mornington Island in the Gulf of Carpentaria, far from the area in Tasmania to which the species is restricted. The original specimen has not been studied but its identity is clear from Labillardiere's plate 183,

and from the Herbarium Webbianum specimen labelled "*Chrysocoma reticulata*" at Kew.

24. *Helichrysum buftoni* sp. nov.

Holotype.—One mile N. of Middleton, *R. Melville*, 2417, 16.xii.1952 (KEW).

Affinis *H. reticulato* sed tomento lanugino crispato non araneoso, foliis minoribus, achaenis glabris differens.

Frutex, rami lanuginosi tomentosi. *Folia* linearia, 1.5–2.5 cm longa, crassa, supra glabrescentia, ruganti in siccitate, subter tomentosa, marginibus revoluta. *Corymbae* terminales densae. *Capitula* numerosa, breviter pedunculata, urceolata vel campanulata, 6–8 mm longa. *Squamae extimae* sparse tomentosae, supra scariosae, glabrescentes. *Laminae squamarum intimarum* fimbriatae obtusae albae patentiores. *Florulae* numerosae. *Achaenia* glabra angulata, pappi setae crassatae in apice.

Shrub 1–2 m high with erect branches bearing terminal inflorescences. Tomentum a tangle of crisped woolly hairs that are dense on older portions. *Leaves* linear, 1.5–2.5 cm long, tomentose below between the revolute margins, glabrescent and slightly wrinkled above (at least when dry), thick and coriaceous as in *H. reticulatum*, obtuse and the older ones reflexed. *Capitula* in dense corymbose clusters or panicles, numerous 6–8 mm long, urceolate to turbinate-campanulate, the bracts subtending the short peduncles glabrous for at least the upper half. *Outer involucre bracts* sparsely tomentose, the intermediate ones with pale obtuse almost glabrous apices; *inner* with erect or slightly spreading white fimbriate but obtuse laminae, the slender claws membranous and villous but stiff and glabrous below. *Florets* more than 30. *Achenes* glabrous angular, pappus bristles conspicuously thickened above.

This species lies between *H. reticulatum* and *H. gunnii* subsp. *paralium*. From the former it differs in tomentum, size of leaves, and the glabrous or almost glabrous achenes, from the latter in the coarser habit, type of tomentum, and the more numerous florets.

The Bufton specimen at Kew seems to have had an involved history. It was forwarded in 1897 and in a letter attached to the sheet Bufton states that he has brought it to England from Tasmania and that Baron von Mueller had proposed to name it after its collector but had never published any description. Its description was requested as Bufton was proposing to prepare a paper for the Linnaean Society on the "Plants of Tasman's Peninsula". Also attached to the sheet is a latin description prepared by Hemsley using Mueller's proposed name. There is no evidence that this was ever published—presumably Bufton failed to issue his paper as it cannot be located. The species appears to be limited to Tasmania.

Apart from the type material the following specimens have been examined:

TASMANIA: Birch's Bay, *L. Rodway* 60, 3.i.1931; sine loc., *J. Bufton*, 1897; St. Paul's Bay, *Avoca*, *R. Gunn* 1167 (KEW); Flower Pot Channel, sine leg. (HO).

25. *Helichrysum stirlingii* F. Muell. in Vict. Nat. 6: 166 (1890).

Lectotype.—Near Mt. Hotham, Australian Alps, *C. Frost*, 1890 (MEL).

Shrub to 3 m high with spreading branches that are tomentose and slightly resinous when young. *Leaves* lanceolate, 4–8 cm long, 8–15 mm wide, 3-nerved owing to submarginal lateral nerves, green but thinly villous with appressed hairs above, pale with a dense but flat tomentum below, the midrib and margins dark in the lower tomentum. *Capitula* 6–15 in terminal corymbs, pedunculate, hemispherical-campanulate, 6–7 mm long, the peduncles subtended by minute tomentose bracts that are almost glabrous at the tips. *Florets* more than 50. *Outer involucrel bracts* ovate, with thin margins, more thinly tomentose than the peduncles, often reddish at the tips; *inner* with conspicuous radiating white laminae, medianly membranous and with woolly hairs, broad and indurate below. *Achenes* papillose, the pappus bristles slender but thickened above. (Fig. 7, A–A₄.)

A species of the southern Dividing Range and extending from southern New South Wales to Mt. Hotham in Victoria.

THE ARGOPHYLLUM COMPLEX

The following three species are very closely related and all come under *H. ferrugineum* sensu Benth. It would have been possible to treat them as subspecies and, indeed, this was seriously considered. However, Wakefield (Vict. Nat. 68: 49 (1951)), being more familiar with them in the field than is the present author, gives them full specific status and since the cytotaxonomy is still unknown it has been decided to leave things as they already are. Nevertheless the distribution suggests that they will be found to form a series similar to that under *H. ledifolium*.

26. *Helichrysum argophyllum* (A. Cunn. ex DC.) N. A. Wakefield in Vict. Nat. 68: 50 (1951); *Cassinia argophylla* A. Cunn. ex DC., Prod. 6: 155 (1838); *Helichrysum ferrugineum* sensu Benth., Fl. Austral. 3: 631 pro parte non Less. (1832) nec Persoon (1807); *Helichrysum ferrugineum* var. *gravesii* (Rodway) Willis in Vict. Nat. 58: 164 (1942).

Holotype.—"In exposed situations on rocky hills at the Illawarra district near Port Jackson, Oct. 1818, Lat. 34½"—A. Cunningham (G-DC).

Haptotype.—"Shaded woods on the ridge of the mountain belt in the west, Oct., 98/1818, 102/1818, N.S.W. 34½ lat."—A. Cunningham (KEW, MEL).

Shrub to 2 m high with densely tomentose grey or rusty branchlets which retain their vestiture longer than is the case in the following two species. *Leaves* linear to lanceolate, 2–7 cm long, glabrous or sparsely villous above, with a close tomentum below in which the dark line of the margin and the midrib are conspicuous and the thinner line of the submarginal laterals (if present) less so. *Capitula* numerous in short dense corymbs terminal to the branches, 3–4 mm long, narrow when very young but spreading in flower and the innermost bracts soon deciduous. *Outer involucrel bracts* with sparse woolly hairs, indurate towards the base but with membranous margins, the extreme edge broken or ragged above more or less transverse wrinkles; *inner bracts* with radiating white laminae about half as long as the claw which is thickened below and bears a few loose hairs. *Florets* 10–18. *Achenes* angular, papillose-hairy, pappus bristles slightly thickened above.

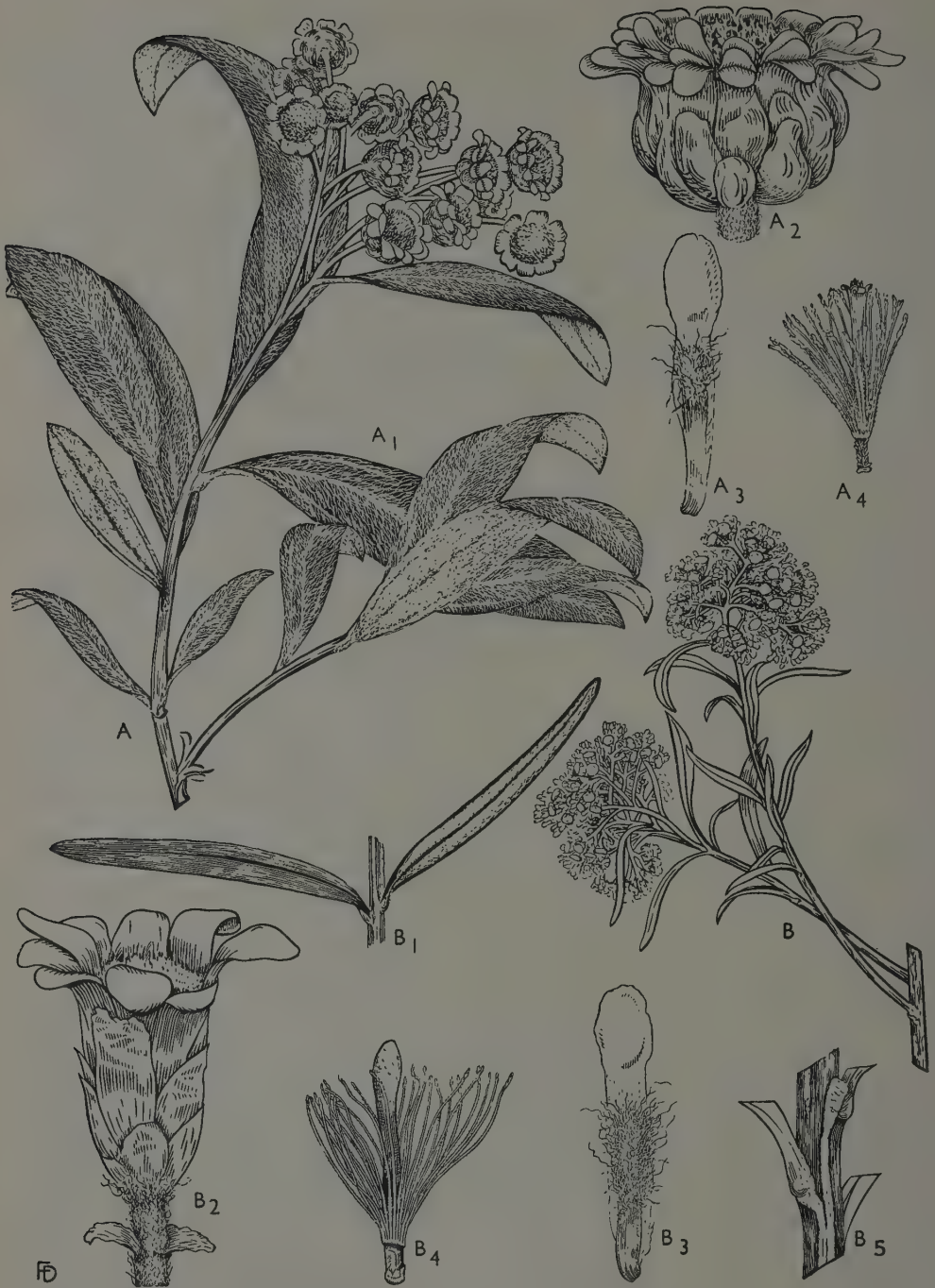


Fig. 7.—A–A₄, *H. stirlingii* F. Muell. (drawn from Moore 2251). B–B₄, *H. dendroideum* N. A. Wakefield (drawn from Burbidge 3028). Lettering as in Fig. 1 but A₂–A₄ all $\times 5$, B₁ $\times 5$, B₂–B₅ $\times 10$.

The data concerning the holotype and haptotype specimens were obtained from the labels attached to sheets from Geneva and Kew respectively. The former is known from a photograph and a fragment and is a very immature specimen, which may explain why Decandolle gives the involucre as 3-flowered. The Kew specimen is slightly over-mature and the involucre is loose owing to the deciduous character of the bracts. It is matched by the sheets at Melbourne, both of which bear the number 102 in Cunningham's hand though the locality is merely given as Illawarra.

The species extends from the south coast of New South Wales through eastern Victoria to Tasmania. According to Wakefield (loc. cit.) it has a more coastal distribution than either of the two following species.

27. *Helichrysum conditum* N. A. Wakefield in Vict. Nat. 68: 50 (1951).

Holotype.—Pine Hill, Suggan Buggan, Vic. (on sandy slopes), Jan. 16, 1948, J. H. Willis (MEL).

Shrub to 3 m high, aromatic and somewhat viscid like the preceding and succeeding species, the stems white tomentose. *Leaves* linear-lanceolate, 1.5–2.5 cm long, 2–3 mm wide, green and glabrous or with a few loose hairs above, pale with a flat tomentum in which the midrib and margins are conspicuous below. *Capitula* in corymbose panicles as in *H. argophyllum*. *Involucre*s 3.5–4 mm long, the bracts approaching those of *H. argophyllum* but less wrinkled, the intermediate ones with stiff pale or whitish tips and the laminae of the inner ones distinctly ovate instead of suborbicular. *Florets* about 16 (Wakefield). *Achenes* papillose-pubescent, the pappus bristles fine but thickened towards the tips.

The only undoubted specimen that the author has seen is the type. While it can be recognized when a suite of material is available it would prove more difficult by itself. However, the hard white apices of the intermediate bracts of the involucre are missing in both *H. argophyllum* and *H. dendroideum* and serve as the most obvious diagnostic character. Wakefield compares it with *H. thyrsoides*. In this case the panicles may be associated with lateral branches rather than being definitely terminal but this feature is not obvious from the material seen.

In distribution it appears to be restricted to drier areas than either of the other two species. Wakefield states that it extends from eastern New South Wales through eastern Victoria to Tasmania.

28. *Helichrysum dendroideum* N. A. Wakefield in Vict. Nat. 68: 50 (1951); *Webbia* 9(2): 467 (1954); *Helichrysum ferrugineum* sensu Benth., Fl. Austral. 3: 631 (1867) pro parte; *H. ferrugineum* (Labill.) Less. ex Steud., Nom. Bot., 2nd Ed. (1840) non Less. (1832) nec Persoon (1807); *Eupatorium ferrugineum* Labill., Pl. Nov. Holl. 2: 38.t.180 (1806); *Chrysocoma ferruginea* Spreng., Syst. 3: 424 (1826); *Ozothamnus ferrugineus* DC., Prod. 6: 165 (1838); *Petalolepis ferruginea* Cass. in Dict. des Sc. Nat. 39: 195 (1826).

Holotype.—"In capite Van-Diemen" (Herb. Webbium ex Herb. Labillardiere-FI) (isotype KEW).

Shrub or small tree to 5 m high, tomentose on very young branches but the vestiture soon restricted to some of the striations, particularly those decurrent to

the leaves. *Leaves* narrow-linear to linear-lanceolate, 2–5 cm long, 2–4 mm wide, glabrous and green above, pale or pale-lemon with a close flat tomentum below apart from the conspicuous midrib and the slightly recurved and often undulate margins. *Capitula* numerous in dense terminal corymbs as in the two species above but the outer and intermediate bracts less wrinkled and not ragged at the apices, the innermost bracts with radiating white shortly oblong laminae. *Florets* rarely more than 6. *Achenes* angular, glabrous or slightly papillose-hairy, pappus bristles scarcely thickened above. (Fig. 7, *B–B*₅.)

Owing to the immaturity of the Cunningham specimen of *H. argophyllum* at Geneva, as shown by the photograph and fragment of the type, the writer while at Kew was inclined to disagree with Wakefield's conclusions regarding the material described under *H. ferrugineum* in the "Flora Australiensis". Wakefield separates *H. argophyllum* on the basis of its denser tomentum, flat (not crenulate) leaf margins, and the broader capitulae with more numerous florets. Few of the Kew specimens appeared to have anything like the 14 florets mentioned by Wakefield and the differences in tomentum and in the involucre bracts were not clear from his text. However, study of specimens in the Melbourne Herbarium, sorted by Wakefield, and of other material has shown that he is correct in recognizing two entities, but this opinion is dependent on accepting the view that Decandolle erred in describing the florets in his *Cassinia argophylla* as being three in number. Accordingly *H. dendroideum* may be recognized by the thinner tomentum which persists on the stem striations, by the minutely undulate leaf margins, by the entire (not thinly ragged) apices of the outer involucre bracts, and by the 4–6 florets per capitulum (not 10–18).

According to Wakefield the species extends from southern New South Wales to Tasmania and also to South Africa. It is a woodland or forest plant and ecologically appears to occupy habitats intermediate between those of the two above.

29. *Helichrysum gunnii* subsp. *gunnii*.

Helichrysum gunnii (Hook. f.) F. Muell. ex Benth., Fl. Austral. 3: 630 (1867); *Ozothamnus gunnii* Hook. f., Fl. Tasm. 1: 205 (1856); *Helichrysum bracteolatus* (Hook. f.) Benth., Fl. Tasm., loc. cit., 203; *Ozothamnus bracteolatus* Hook. f., Fl. Tasm., loc. cit., 203.

Holotype.—Sandhills, seashore George Town 27.i.1843, Gunn 1256/1842 (KEW) (photograph No. 1595, CANB).

Holotype of Synonym.—Flinders Island, Gunn 497/1842 (KEW).

Slender erect shrub to 2 m high, branches and branchlets very softly arachnoid-tomentose. *Leaves* linear and revolute on the margins (linear-lanceolate and recurved in *H. bracteolatus*), channelled above by the midrib and sparsely webby or subglabrous; the lower surface where exposed densely tomentose like the stems, from 1 cm long on short axillary shoots (immature) to 3.5 cm along the stem and 2–3 mm wide. *Capitula* in dense corymbs terminal to the stems. Involucres 4–5 mm long (longer in *H. bracteolatus*?), 8–12-flowered, the outer bracts arachnoid-tomentose, the intermediate less so, the innermost with conspicuous crumpled milky white laminae orbicular in outline and wider than their claws. *Achenes* papillose to pubescent, the pappus bristles slightly thickened above.

I am unable to accept Wakefield's view in *Webbia* 9(2): 468 (1954) where he includes *Ozothamnus turbinata* as a synonym. However, it must be admitted that there are specimens which might prove difficult to place in the absence of a good assembly of material and consequently a compromise has been reached by the establishment of two subspecies.

It is unfortunate that *H. bracteolatus*, which is known only from the by no means abundant material of the original type, should by having somewhat larger involucre and more numerous florets bring the description of subsp. *gunnii* nearer that of subsp. *paralium*. The fact is that in general aspect the type specimen of *H. bracteolatus* does not in the least approach subsp. *paralium*, since it has the webby vestiture of subsp. *gunnii* with broader leaves recurved along the margins, quite different from the closely revolute partially resinous ones of subsp. *paralium*. The additional fact that these broader leaves are on a vegetative shoot, lateral to that terminating in the single corymb, increases the likelihood that this specimen represents an atypical individual rather than a distinct taxon. The corymb also differs in that only the terminal capitula are developed though the bracts which normally subtend all involucre are present.

No specimens of subsp. *gunnii* from the Australian mainland have been seen so that it appears to be confined to Tasmania.

29a. *Helichrysum gunnii* subsp. *paralium* nom. et stat. nov.

Helichrysum gunnii sensu Wakefield in *Vict. Nat.* 68: 51 (1951) et in *Webbia* 9(2): 468 (1954) pro parte; *Helichrysum cinereum* sensu Benth., *Fl. Austral.* 3: 629 (1867); *Ozothamnus cinerea* Hook. f., *Fl. Tasm.* 1: 203 (1856) sed non *Chrysocoma cinerea* Labill., *Nov. Holl. Pl.* 2: 39 (1806); *Ozothamnus turbinatus* DC., *Prod.* 6: 164 (1838).

Holotype.—"Nouv. Hollande cote Merid., Mus. de Paris 1821" (G-DC) (= holotype of *O. turbinata*).

Shrub to 3 m high, the stems with close white or yellowish tomentum which is tightly woolly rather than webby though looser on young shoots. *Leaves* narrow linear, closely revolute below, 1–2.5 cm long, 1–1.5 mm wide, glabrous or sub-resinous above and channelled along the midrib, tomentose below and usually with a patch of bright yellow exudate at the base. *Capitula* in terminal corymbs, 6–7 mm long, 15–20-flowered. *Involucre*s turbinate, the outer bracts woolly on the margins rather than the dorsal surfaces and slightly resinous, intermediate bracts woolly, innermost with small crumpled white laminae not specially wider than their claws. *Achenes* minutely papillose.

This is distinguished from subsp. *gunnii* by its woolly rather than webby tomentum, the much narrower leaves with their characteristic patches of yellow exudate at the base, and the larger capitula with more numerous florets.

It has been clearly shown by Wakefield (1951) that Labillardiere's *Chrysocoma cinerea* was based on a New Caledonian plant and that the epithet has been wrongly applied to Tasmanian and Victorian material. He later (1954) published further comments after having the original checked in Florence. In the meantime the

present author had attempted to trace a Labillardiere specimen in London. That such a specimen should exist seemed likely from Hooker's remarks in the "Flora Tasmaniae", where he says: "Labillardiere's original specimens differ from Gunn's only in the rather shorter leaves and their slightly more tomentose upper surface". The Gunn material referred to here is not that used for the type of *H. gunnii*.

At Kew there is a specimen from Herbarium Hookerianum with a label "*Chrysocoma cinerea* t.182". There is nothing to indicate that this is from Labillardiere's herbarium but the quotation of the original plate may be significant. The specimen agrees closely with the original description of *Chrysocoma cinerea* including "semina obovata, piluscula", and also agrees with authentic material of *H. neocaledonicum* Schlecht. at the British Museum (Natural History), thus independently confirming Wakefield's opinion.

Also at the British Museum there is a sheet labelled "*Chrysocoma cinerea*" in an unknown hand to which has been added "ex Herb. Labillardiere" by Spencer Moore. This specimen is not the same as *H. neocaledonicum* but is conspecific with Tasmanian material collected by David Nelson, Robert Brown, Gunn, and others and determined as *H. cinereum*. It seems probable that this is the same as the Labillardiere sheet "a" discussed by Wakefield (1954).

Material named as *H. cinereum* by Bentham and quoted in the "Flora Australiensis" was compared with a photograph and with fragments of the type of *Ozothamnus turbinata* DC. and there can be little doubt as to their conspecificity. Neither the type nor the Kew specimens closely resemble the type of *H. gunnii* Hook. f. Unfortunately the epithet "*turbinata*" cannot be employed because it has already been twice applied to Australian species of *Helichrysum*. The epithet "*paralium*" has been selected as indicative of the consistently coastal distribution.

It is common on sand dunes in Tasmania and extends to Victoria and South Australia, being represented in the Melbourne Herbarium by the following: Lorne, Vic., A. C. F. Gates, 6.i.1922; Rivoli Bay, S.A., Mrs. Wehl; as well as by other Victorian specimens.

30. *Helichrysum rosmarinifolium* (Labill.) Steud. ex Benth., Fl. Austral. 3: 631 (1867); *Eupatorium rosmarinifolium* Labill., Nov. Holl. Pl. 2: 38 (1806); *Petalolepis rosmarinifolium* Cass., Dict. des Sc. Nat. 39: 195 (1826); *Chrysocoma rosmarinifolia* Spreng., Syst. Veg. 3: 424 (1826); *Ozothamnus rosmarinifolius* DC., Prod. 6: 165 (1838) et Hook. f., Fl. Tasm. 1: 205.t.54 (1856).

Lectotype.—"In Capite Van-Diemen" (Herb. Webb. ex Herb. Labillardiere-FI).

Shrub to 2.5 m high, tomentose on young branches but less so when old. *Leaves* linear, 1.5–4 cm long, closely revolute on the margins; upper surface muricate-tuberculate and very scabrid, channelled along the midrib, lower surface tomentose. *Capitula* numerous in corymbose panicles terminal to the main and upper branchlets, narrow-campanulate, 3.5–4.5 mm long. *Outer involucrel bracts* scarious, almost glabrous though the intermediate ones with some basal hairs, obtuse and slightly transversely wrinkled in the upper halves which are often reddish; *inner bracts* with white radiating laminae that are crumpled on the margins

and thin sparsely hairy claws that are thickened towards the base. *Florets* 5-7. *Achenes* glabrous, pappus bristles scarcely thickened above. (Fig. 5, *A-A*₄.)

The identity of this species has been fully discussed by Wakefield (Vict. Nat. 68: 51 (1951); *Webbia* 9(2): 466-8 (1954)) and this has greatly clarified the confusion resulting from Bentham's composite description. It is widespread in Tasmania and extends through Victoria to south-eastern New South Wales.

THE LEDIFOLIUM COMPLEX

With the elucidation of *H. rosmarinifolium*, *H. thyrsoides*, and *H. secundiflorum* by Wakefield the most complicated group remaining is that centred around *H. ledifolium* (DC.) Benth., *Ozothamnus purpurascens* DC., and *H. alpinum* Wakefield. The first two are based on plants from Tasmania and the last on one from Mt. Hotham in Victoria, but together they appear to be members of a single complex. Furthermore the variation in the field has resulted in confusion producing a nomenclatural tangle in the literature.

In his "Prodromus", Decandolle described both *Cassinia ledifolia* and *Ozothamnus purpurascens*, the first being based on specimens collected on Mt. Wellington by Allan Cunningham and the latter on one without locality collected by Gunn and forwarded to Decandolle by Lindley. Gunn's specimen is numbered 281 and Lindley apparently forwarded it in 1834. Unfortunately there are no specimens of Gunn 281 of such an early date at Kew though there are some later collections so numbered. These were placed under *H. ledifolium* (DC.) Benth. for the "Flora Australiensis". However, what appeared to be Gunn 281/1834 was located in the Lindley Collection at Cambridge though the treatment, both in Bentham's "Flora Australiensis" and in Hooker's "Flora Tasmaniae", of *O. purpurascens* make it doubtful if either of these authors had critically examined the Lindley Herbarium specimen.

Hooker's confusion of *O. purpurascens* DC. with *Eupatorium rosmarinifolium*, which was repeated by Bentham, has only recently been clarified by Wakefield, who in his treatment gives *O. purpurascens* as a synonym of *H. ledifolium* though no argument is provided.

After studying a photograph and a fragment of the types received from Geneva and also the material at Cambridge the author has reached a conclusion intermediate between that of Decandolle and of Wakefield, i.e. that while *O. purpurascens* is synonymous with *H. ledifolium* sens. lat. it represents a different taxon to that of the holotype of *H. ledifolium* sens. str. and that the difference is best dealt with under subspecific status.

Among the specimens at Kew named by Bentham as *H. ledifolium* is one collected by Mueller in the Munyang Mts. and labelled by him as *Ozothamnus purpurascens*. This undoubtedly approaches the plant used as a type by Decandolle but it seems to represent the mainland variant of the complex. The leaves approach those of *H. secundiflorum* but the habit of branching and the immature capitula are different. It therefore agrees better with *H. alpinum* Wakefield, if his reference to *H. hookeri* var. *expansifolium* be excluded, though he described the leaves as

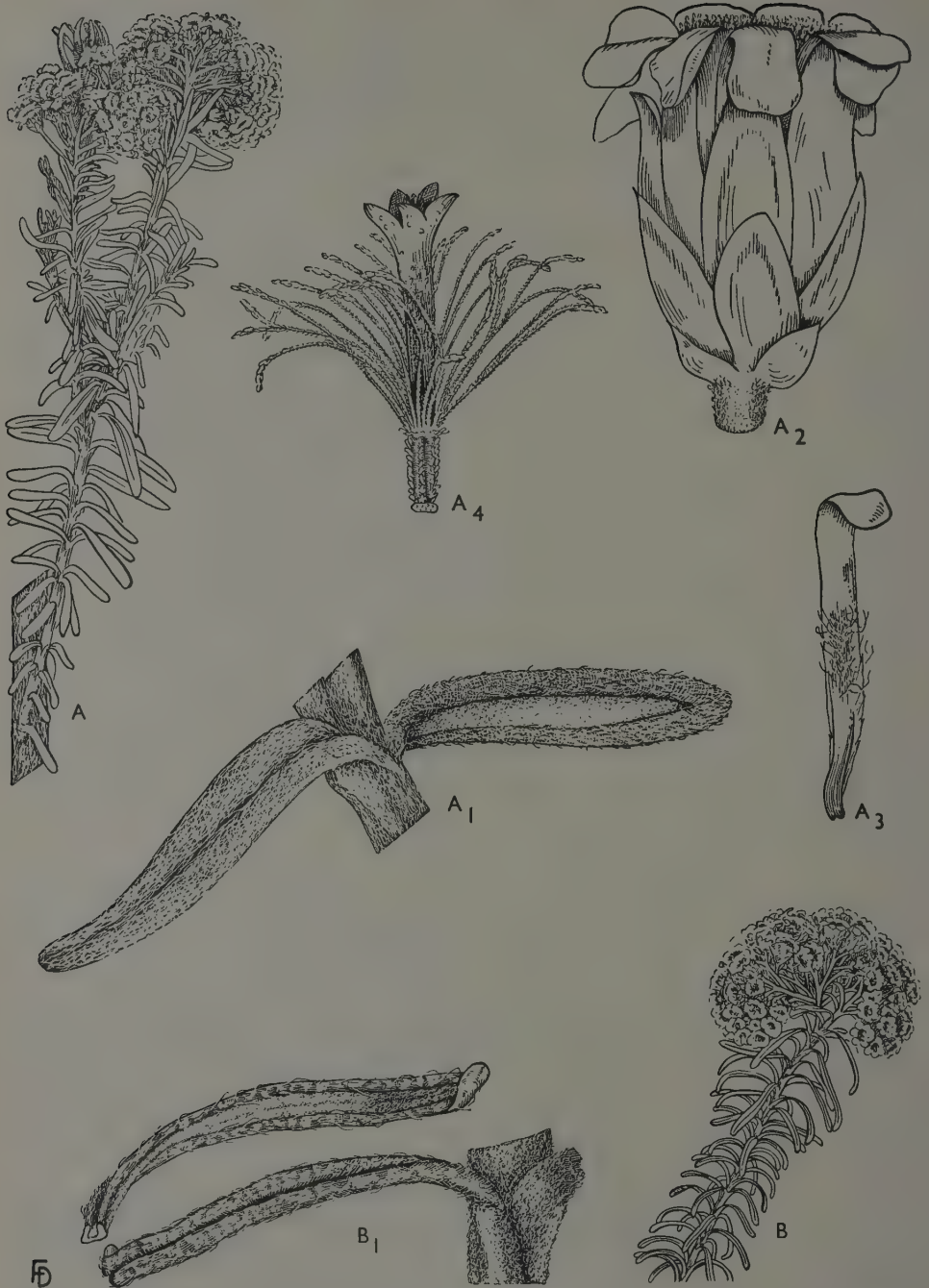


Fig. 8.—A–A₄, *H. ledifolium* subsp. *ledifolium* (drawn from Burbidge 3187). B–B₁, *H. ledifolium* subsp. *purpurascens* (DC.) comb. nov. (drawn from “Huon rd. to Mt. Wellington, C. E. Lord”). Lettering as in Fig. 2.

glabrous above. Inspection of the Tasmanian material of *H. ledifolium* sens. lat. shows that some hairs may be present on the upper surface but that a resinous exudate normally obscures them, while on a specimen from Mt. Kosciusko (Burbidge 3758), which has been checked as *H. alpinum* by Wakefield, the young leaves bear appressed webby hairs but older ones are resinous and apparently glabrous at first glance.

Another name which has been linked with that of *H. ledifolium* is *Ozothamnus ericifolius* Hook. f. (see Plate 57 in "Flora Tasmaniae") and Rodway in describing *O. rosmarinifolius* var. *ericifolius* evidently had this in mind though he omits any cross reference (Tasm. Fl. (1903)). However, in the writer's opinion the taxon which Rodway used was not *O. ericifolius* Hook. f. but *O. purpurascens* DC. which differs from Hooker's plant in the longer leaves, less floriferous habit, larger capitula, and much more extensive distribution. It should also be noted that Hooker's name is not concerned with the same taxon involved in the combination proposed by Cunningham and quoted by Decandolle under his *O. diosmaefolius*.

The groups of specimens which result from the sorting of herbarium material under a strict application of the names involved also result in a sorting into ecological habitats, e.g. the plants which come under *H. ledifolium* sens. str. are to be found on the upper slopes and summit of Mt. Wellington while those under *O. purpurascens* appear to be restricted to the lower slopes below 1000 ft elevation. A local study of the variation in the field will be necessary before the true relationship can be understood. In the meantime they have been treated as subspecies.

31. *Helichrysum ledifolium* subsp. *ledifolium*

Helichrysum ledifolium (DC.) Benth., Fl. Austral. 3: 630 (1867); *Cassinia ledifolia* DC., Prod. 6: 155 (1838); *Ozothamnus ledifolius* Hook. f., Fl. Tasm. 1: 204 (1856).

Holotype.—"103. a stunted shrub found on the more elevated parts of Mt. Wellington near Hobart Town, Van Diemen's Land, Jany. 1819"—A. Cunningham (G-DC).

Shrub to 1 m high with a rigid corymbose habit of growth, the older stems marked by the corky persistent bases of the leaves. Younger branches and leaves tomentose, often bright yellowish green when dry. *Leaves* linear, thick, revolute on the margins, 7–15 mm long, 1.5–2 mm in diameter, the midrib slightly sunken in the thinly tomentose or subglabrous upper surface but obscured by tomentum below. *Corymbs* terminal, the bracts subtending the capitula ovate, acute, villous on the back, and frequently with a few long hairs projecting at the apices. *Involucre*s turbinate, 5–7 mm long; *outer bracts* loosely rather than densely tomentose; *innermost* 4.5–5 mm long, the claws almost linear and villous in the upper half though glabrous and indurate below, the white laminae almost orbicular. *Florets* 9–12. *Achenes* papillose-pubescent, the vestiture appearing to form a minute ring around the base of the pappus. (Fig. 8, A–A₄.)

This comparatively coarse looking form seems to be confined to high altitudes and to mountain habitats. The majority of the specimens at Kew are from Mt.

Wellington but there is a Lawrence specimen from "Western Mountains 3000 feet" and also "National Park near Lake Fenton, *A. W. Hill*, 15.i.1925".

31a. *Helichrysum ledifolium* subsp. **purpurascens** stat. nov.

Ozothamnus purpurascens DC., Prod. 6: 165 (1838); *O. rosmarinifolius* var. *ericifolius* Rodway, Tasm. Fl. 89 (1903).

Holotype.—"Van Diemen's Land, *Gunn* 281 ex Herb. Lindley 1934" (G-DC) (isotype—CGE).

Shrub 1–2 m high, of columnar habit of growth with erect main branches and numerous comparatively short laterals. Young stems and under-surfaces of the leaves softly tomentose, upper surfaces slightly resinous, the exudate often concealing the sparse tomentum. *Leaves* linear, revolute or much recurved on the margins, acute or obtuse, 7–18 mm long, less than 1 mm thick when revolute, spreading to reflexed especially when old, channelled above, the bases of the old leaves not conspicuous on the older stems. *Corymbs* terminal to the lateral and main branches. *Bracts* subtending the involucre ovate, acute-acuminate, the scarious apices often glabrous. *Involucre*s similar to those of subsp. *ledifolium* though the innermost bracts may be almost glabrous and the florets 8–10. (Fig. 8, *B–B*₁.)

The plants are much less massive in habit than in the above. Judging from the specimens seen they are found at low altitudes between 300 and 800 ft especially in the Hobart area. Among these were the following:

Specimens Studied.—TASMANIA: Huon Road, *F. Rodway*, Dec. 1902 (MEL); do., *H. D. Gordon*, 1.xi.1937 (HO); do., *C. E. Lord*, January 1930 (KEW, CANB); Huon Road near Hobart, on dry ridge 800 ft, *H. F. Comber*, 26.i.1930; Mt. Wellington in open exposed places 800 ft, *H. F. Comber* 1595, 13.xi.1929 (KEW); Longley, *A. V. Giblin* (KEW, CANB); Cradle Mt., *G. Wiendorfer* (MEL).

31b. *Helichrysum ledifolium* subsp. **reflexum** subsp. nov.

Affinis subsp. *purpurascens* sed plantis tomentosis, foliis juvenalis reflexibus differens.

Holotype.—Sea cliffs at Remarkable Cove, $\frac{1}{2}$ mile S. of Safety Cove, Tasman Peninsula, *R. Melville* 2500, 18.xii.1925 (KEW).

Shrub 1–2 m high growing near the coast, the branching tending to be whorled owing to groups of shoots developing immediately below the old terminal corymbs. Tomentum loosely woolly on all parts though thinner on the upper leaf surfaces. *Leaves* linear, revolute on the margins, obtuse, 3–12 mm long, 1–1.5 mm wide, reflexed or strongly recurved even on the current season's growth. *Corymbs* terminal and densely subglobose. *Involucre*s turbinate to cylindrical-campanulate, 4–5 mm long; the *outer bracts* sparsely tomentose, *inner* 3.5–4 mm long, the narrow claws villous above and membranous except at the base. *Florets* 8–10. *Achenes* not seen mature but similar to those in subsp. *ledifolium*.

This appears to be the coastal variant of the complex and its peculiar branching habit suggests that of *H. selaginoides*. It is closest to subsp. *purpurascens* and might have been included as a form of it though the extremes are very different.



Fig. 9.—A–A₄, *H. alpinum* N. A. Wakefield (drawn from *Burbidge* 3758). B–B₁, *H. ledifolium* subsp. *ericifolium* (Hook. f.) comb. nov. (drawn from *Burbidge* 3418). Lettering as in Fig. 2.

Specimens Studied.—TASMANIA: Flinders Island, *Backhouse* (KEW); mountain near Port Arthur, *J. Bufton* 43, 1892; Port Arthur, *J. Bufton*, 1893 (MEL); Lindisfarne, *A. M. Olsen*, 22.xi.1936 (HO).

31c. *Helichrysum ledifolium* subsp. *ericifolium* stat. nov.

Ozothamnus ericifolius Hook. f. in Hook., Lond. J. Bot. 6: 119 (1847).

Holotype.—Marlborough, 7 ft high, 5.i.1841, *Gunn* 1163/1842 (KEW).

Shrub 1.5–3 m high with columnar growth habit, the tomentum as in subsp. *ledifolius* but apparently not so apt to turn yellowish green. *Leaves* oblong-linear, revolute on the margins, tomentose on both surfaces or glabrescent above, 4–7 mm long, 0.5–1 mm wide, less than 0.5 mm thick, spreading but becoming reflexed with age. *Capitula* clustered at the ends of short lateral branches, the plants very floriferous, subtending bracts ovate, acute, semiscarious, the hairs not projecting beyond the apices. *Involucres* turbinate, 3.5–4.5 mm long; *outer bracts* fewer than in subsp. *ledifolium*, sparsely villous or tomentose; *inner bracts* 3–3.5 mm long, the claws glabrous or with one or two long curly hairs. *Florets* 5–6. *Achenes* as in subsp. *ledifolium* (Fig. 9, B–B₁).

As well as the type there is a Herb. Benthamianum sheet at Kew which bears two specimens. These are: (1) a spray labelled “Tasmania, Antarc. Expedition, 1839–43, J.D.H.” and (2) “Gunn 1163/1844” without locality other than Tasmania. The Hooker specimen has a more open growth and the leaves are glabrescent above. It agrees with the right-hand spray of the three on the type sheet and hence it seems best to regard only two of these as truly holotypic. The second Gunn specimen is probably a later collecting as Gunn was in the habit of repeating his numbers from year to year in the absence of names.

The Hooker specimen mentioned above is also represented in the Melbourne Herbarium. Other specimens include: “Great Lake, *A. Simson* 2336, 1892” (MEL) and “6 miles S.W. of Great Lake on Bronte Rd.”, *N. T. Burbidge* 3418, 27.i.1949 (CANB, HO).

32. *Helichrysum alpinum* N. A. Wakefield in Vict. Nat. 68: 49 (1951).

Holotype.—Mt. Hotham, Vic., *C. Walter*, Jan. 1888 (MEL) (isotype at CANB?).

Alpine shrub to 1.5 m high, the younger shoots with yellowish grey tomentum. *Leaves* oblong, obtuse, 5–7 mm long, 2–3 mm wide, spreading, slightly pubescent above or with appressed hairs appearing neatly brushed out from the midrib or the tomentum wholly obscured by resin, the margins recurved and at first tomentose like the under-surface. *Capitula* in subglobose clusters at the ends of short upper branchlets, 4.5–5 mm long, cylindrical. *Outer involucral bracts* reddish, almost glabrous, thinly scarious towards their apices; *inner bracts* with conspicuous white oblong-obtuse laminae and slender claws thickened at the base. *Florets* 4–6. *Achenes* glabrous, the pappus bristles not thickened above. (Fig. 9, A–A₄.)

This species represents the mainland variant of the *ledifolium* complex and is quite distinct from *H. hookeri* var. *expansifolium* Morris & Willis included by Wakefield in the comments under his description, which is evidently based on

non-Tasmanian specimens. The latter taxon is raised in status and further discussed below.

H. alpinum includes the Mungyang Mts. material which Mueller distributed as *H. purpurascens*, judging by a sheet seen at Kew. It differs from the latter in the smaller flatter leaves, more glabrous involucre, and the fewer florets. It is widespread at high altitudes in the Australian Alps and extends south through the more elevated parts of the ranges in Victoria.

33. *Helichrysum cuneifolium* Benth., Fl. Austral. 3: 633 (1867) non Tovey & Morris (1923); *Helichrysum oblongifolium* Tovey & Morris in Proc. Roy. Soc. Vict. 35: 195 (1923).

Lectotype.—Snowy R., *F. Mueller* (KEW) (photograph No. 1608 at CANB).

Shrub to 3 m high with the branches covered by a flat or almost floccose tomentum. *Leaves* 2–4 cm long, 7–10 mm wide, cuneate, narrowed below into short petioles, 1.5–4 mm long, very obtuse, glabrous above or slightly resinous but with a flat tomentum below in which the midrib is slightly prominent and the recurved margins are crisped or minutely undulate. *Capitula* in spreading corymbs terminal to the upper branches, narrow, cylindrical or narrowly subcampanulate, 4–4.5 mm long. *Outer involucre bracts* slightly and loosely woolly; *inner* with milky white laminae about half as long as their claws which are thickened towards the base and sparsely woolly-villous on the back. *Florets* 4–6. *Achenes* angular, minutely pubescent, the pappus bristles slightly swollen at the tips.

The Snowy River specimen selected as lectotype bears the name "*Ozothamnus cuneifolius*" in Mueller's hand and Bentham in the "Flora Australiensis" writes "*Helichrysum cuneifolium* *F. Muell. Herb.* (as an *Ozothamnus*)". This may be taken as indicating his acknowledgment of Mueller's right of recognition but it hardly seems, under Article 43 of the International Code, a valid publication of the combination under the latter genus. At the same time Bentham's combination under *Helichrysum* is effectively and legitimately formed, under the rules of the period, even though the epithet should have been taken up for and instead of *H. backhousii*. For this reason the name *H. oblongifolium* Tovey & Morris must be regarded as superfluous and their combination *H. cuneifolium* (DC.) Tovey & Morris is a later homonym of Bentham's. (See Morris and Willis in Vict. Nat. 59: 85 (1942).)

The species is distributed from the south coast areas of New South Wales to Gippsland in Victoria.

34. *Helichrysum antennarium* (DC.) *F. Muell. ex Benth.*, Fl. Austral. 3: 632 (1867); *Swammerdamia antennaria* DC., Prod. 6: 164 (1838); *Ozothamnus antennarius* Hook. f., Fl. Tasm. 1: 203 (1856).

Holotype.—Insula Van Diemen, *Gunn* 274 ex Herb. Lindley 1836 (G-DC).

Shrub to 1.5 m high with glabrous branches. *Leaves* oblanceolate to obovate, narrowed below into a slender petiole, glabrous and slightly resinous above, pale below with a conspicuous midrib and slightly recurved margins. *Capitula* in terminal clusters or short corymbs, shortly pedunculate, turbinate-campanulate,



Fig. 10.—A-A₄, *H. backhousii* (Hook. f.) F. Muell. ex Benth. (drawn from Burbidge 3353).
 B-B₄, *H. thyrsoides* (DC.) Willis & Morris (drawn from "Eaglehawk Neck, Giblin").
 Lettering as in Fig. 2.

about 5 mm long. *Outer involucre bracts* oblong, slightly concave, sparsely woolly on the back, the margins thinner and often slightly ragged; *inner* with small white laminae about a third as long as their claws which are sparsely woolly on the back and thickened towards the base. *Florets* 20–25. *Achenes* pubescent, the pappus bristles numerous and thickened at the flattened apices.

A fragment and photograph of the type sheet was received from Geneva. The sheet bears two specimens: (1) Gunn 274 labelled “Van diemen. M. Gunn env. par. Lindley 1836” and (2) “Tasmania ex Herb. Boissier e Museo Parisiensi, Reuter 1846”. Since the species was published in 1838 only Gunn 274 can be regarded as the type. It is over-mature and the involucre are partially fallen whereas in the other specimen they are still complete. It is suspected that the actual material forwarded may have come from this and not from Gunn 274 though its conspecificity is not in question. At Kew there are two specimens bearing Gunn’s number. One reads “No. 274, Van Diemen’s Land, R. Gunn, Sir William Hooker 1838” and the other “No. 274 Van Diemen’s Land Gunn 1835”. The latter is the better match for the Genevan photograph and may represent part of the original collection. The former would, from the date, appear to have been collected too late. In the Lindley Collection at Cambridge there is a Gunn specimen labelled “274/1842 Mt. Wellington” but this too, from the date, cannot be part of the holotype.

The species appears to be confined to Tasmania and to elevations of more than 3000 ft.

35. *Helichrysum backhousii* (Hook. f.) F. Muell. ex Benth., Fl. Austral. 3: 632 (1867); *Ozothamnus backhousii* Hook. f., Fl. Tasm. 1: 204 (1856); *Cassinia cuneifolia* A. Cunn. ex DC., Prod. 6: 155 (1838); *Helichrysum cuneifolium* (DC.) Tovey & Morris in Proc. Roy. Soc. Vict. 35: 195 (1923) non Benth. (1867).

Lectotype.—Mt. Wellington, R. Gunn 1165/1842, 31.i.40 (KEW). (Photographs Nos. 1006, 1006a at CANB.)

Syntypes.—“Summit of Mt. Wellington, Fraser”, also “Port Arthur, Backhouse” (KEW).

Holotype of Synonym.—“A shrub of stunted growth on the rock face of Mount Wellington, A. Cunningham 104, Jany. 1819” (G-DC).

Shrub to 1 m high, the branches yellowish or grey with a sparse obscure tomentum which is slightly resinous when young. *Leaves* obovate to cuneate, 7–10 mm long, very obtuse, green and minutely pubescent or glabrous above, pale below with an appressed tomentum in which the midrib is obscure but the minutely recurved margins are distinct. *Capitula* in subglobose clusters terminal to the upper branches, turbinate to campanulate, 5–6 mm long. *Outer involucre bracts* brown or reddish, sparsely woolly with long tangled hairs, scarious above and sometimes transversely wrinkled, obtuse; *inner* with small white radiating or spreading-erect laminae that are about a third as long as the claw which is membranous above and thickened below, a few hairs being present on the lower portion. *Florets* 12–15. *Achenes* glabrous or minutely papillose, pappus bristles slightly thickened above. (Fig. 10, A–A₄)

The branches are sturdy and thicker than those in *H. antennaria* and the internodes are consistently shorter. It is a species of alpine areas in Tasmania and is found at higher elevations than is usual for the latter species. The inner involucral bracts are soon deciduous and are often few or inconspicuous in herbarium material.

The nomenclature of this species has been mentioned under *H. cuneifolium*. Since Bentham, having already used the epithet "*cuneifolium*", obviously could not repeat it he was correct in applying the next valid one. An exactly parallel case occurs with *H. hookeri* (Sond.) Druce and *H. lepidophyllum* (Steetz) F. Muell. ex Benth.

The sheet at Kew which bears Hooker's pencil sketches has material of three collections which have all been quoted above. In the "Flora Tasmaniae" he mentions Gunn 1165 at the end of the description and then goes on to cite the remaining material. Since he did this it is presumed that Gunn's specimen was basic to his description though there is evidence that the sketches are based on all three.

Specimens Studied.—TASMANIA: Mt. Olympus, *E. D. Briggs*, 6.i.1928 (NSW); Mt. Rufus, Lake St. Clair, *N. T. Burbidge* 3353, 25.i.1949 (CANB, HO); Mt. Field East, *J. H. Maiden*, March 1906 (NSW); Wombat Moor, National Park, *F. H. Long* 1177, 26.iii.1932 (HO); Mt. Wellington, *Gunn* 1165/1842; do., *A. Cunningham* 60, 8.i.1819; do., *Fraser* (KEW); do., *L. Rodway*, June 1900; also *P. Helms*, 19.i.1902, and *A. H. S. Lucas*, Jan. 1901 (NSW).

There is also an atypical specimen collected by Rupp from Mt. Barrow in January 1922. This is very grey of aspect and softly tomentose on the upper surfaces of the leaves which are smaller than is usual in this species. Some features of the specimen suggest an affinity with the *ledifolium* complex and in the absence of additional material it can only doubtfully be placed under *H. backhousii*. The specimen is held in the Herbarium at the Sydney Botanic Gardens.

36. *Helichrysum thyrsoideum* (DC.) Willis & Morris in Vict. Nat. 59: 86 (1942);
Ozothamnus thyrsoideus DC., Prod. 6: 165 (1838) and Hook. f., Fl. Tasm.
1: 205 (1856).

Holotype.—Van Diemen's Land, *Gunn* 240, ex Lindley 1836 (G-DC).

Shrub to 3 m high, the branches striate-angular, pubescent especially on the decurrent striae below the leaves. *Leaves* narrow linear, 2–5 cm long, 1.5–2 mm wide, obtuse to acute, dark green and minutely wrinkled above (when dry) and slightly resinous though apparently glabrous, pale below with appressed tomentum except for the conspicuous midrib and margins. The upper branches highly floriferous with numerous clusters of 10–20 capitula at the ends of short laterals along the main spreading branches and thus forming long secund inflorescences. *Capitula* turbinate-campanulate, 4–5 mm long. *Outer involucral bracts* pale or brownish, woolly, scarious to chartaceous; *inner* with conspicuous radiating white laminae about half as long as the stiff claws which have membranous margins and bear a few long hairs. *Florets* 10–16. *Achenes* papillose-hairy, the pappus bristles distinctly thickened above. (Fig. 10, B–B₄.)

The white laminae of the inner bracts are very conspicuous immediately prior to and during flowering but later many of them fall and the spreading pappus bristles obscure them. The long floriferous sprays make this one of the most handsome species in the subgenus.

The holotype material at Geneva was collected from a plant in an early flowering state, the involucre not showing any signs of breaking up. The same number, i.e., Gunn 240, is represented at Kew by Gunn 240/1842 collected in the Western Mountains at an altitude of 3600 ft on 18.ii.1843. This specimen, which could not possibly have reached Europe until years after the publication of the "Prodromus", was evidently obtained at the full height of flowering as the involucre in drying have opened wide and the pappus expanded.

The species is widespread in Tasmania where it occurs on the lower hills as well as on the mountain slopes. It extends through Victoria into south-eastern New South Wales and the Australian Capital Territory.

Two specimens have been examined which may represent either distinct taxa or hybrids between *H. thyrsoides* and some other species, possibly *H. stirlingii*. In Melville 2964 from "Upper Delegate R. nr. Bidwell, Gippsland, Sclerophyll forest near margin of swamp, 19.i.1953" (KEW) the general habit is very similar, the leaves being 5–25 mm long and 1–1.5 mm broad, but the margins are more revolute. The involucre are campanulate-hemispherical and 20-flowered and the pappus bristles are numerous. The possibility of describing this under a varietal name was considered but the existence of another suspected hybrid shows that the situation requires further investigation. The second specimen is one collected by H. C. E. Stewart on Mt. Buffalo at 4500 ft, 5.i.1950, and held in the herbarium at Brisbane. It has heads intermediate between those of *H. thyrsoides* and of Melville 2964 but the leaves are elliptical-lanceolate, 2–6 cm long, and with the tomentum of *H. secundiflorum*. It is hoped that field and other studies will demonstrate the status of these forms, but for the present it does not seem advisable to do more than to note their existence.

37. *Helichrysum secundiflorum* N. A. Wakefield in Viet. Nat. 68: 49 (1951).

Holotype.—Cobboras Mts. (6000 ft), Vict., Feb. 1854, *F. Mueller* (MEL).

Shrub to 2 m high with striate tomentose branches. *Leaves* linear-oblong to very narrow cuneate, 8–10 mm long, about 1.5 mm wide, tomentose above with webby hairs, densely and softly tomentose below, the midrib and margins evident but not conspicuous. The main lateral branches very floriferous as in *H. thyrsoides*. *Capitula* in clusters along and at the ends of short laterals, campanulate to turbinate, 3.5–4 mm long. *Outer involucral bracts* brown, scarious-chartaceous, sparsely woolly; *inner* with conspicuous white radiating laminae about half as long as their stiff claws which bear a few long hairs on the back. *Florets* about 15. *Achenes* papillose-hairy, the pappus bristles scarcely thickened above.

According to Wakefield this species is particularly associated with the Australian Alps but it extends north to the Jenolan Caves in the Blue Mountains of New South Wales and south to Wilson's Promontory in Victoria.

38. *Helichrysum selaginoides* (Sond. & F. Muell.) F. Muell. ex Benth., Fl. Austral. 3: 634 (1867); *Ozothamnus selaginoides* Sond., & F. Muell. in Linnaea 25: 510 (1853).

Holotype.—There is a sheet at Melbourne which must be regarded as that from which the type was taken. Presumably the material used by Sonder is in the Sonder Collection at the Naturhistoriska Riksmuseum in Stockholm but it is not known which of the three pieces on the Melbourne sheet should be classed as isotypic. There are three labels, one bears the name only, one has the name and "Table Mountain Mar./49", and the third "*Ozothamnus* sp. Table Mountain 3000 ft., 1–2 ft., Sept. 28th" with the name written in full by Mueller at a later date.

Shrub to 1 m high, the branches covered by the adpressed bases of the numerous leaves. *Leaves* oblong, obtuse and rather thick, 2.5–3.5 mm long, the upper half recurved and divergent to the stem, glabrous and slightly viscid. *Capitula* sessile narrow-turbinate, 4–5 mm long, in small terminal clusters but lateral from the axils immediately below often developed during flowering and thus surrounding the inflorescences. *Outer involucrel bracts* scarious-chartaceous, slightly viscid but with woolly hairs, membranous at the apices and along the margins; *inner* with small white radiating laminae about a third as long as the glabrous claw which is membranous above but thickened towards the base. *Florets* 8–12. *Achenes* papillose, the pappus bristles thickened at the tips.

On the Kew sheet of Gunn 1975/ "Table Mountain W. of Oatlands, 1/11/45" the coherent anthers protrude and are quite conspicuous. This is less evident in the Stuart material at Melbourne on which the white laminae of the bracts have irregular or ragged margins.

Table Mountain is an old name for Mt. Wellington but according to recently received information, after 1837 the latter name was accepted and Gunn's locality was the Table Mountain in the Lake Sorell area which is still the only place for which the species has been recorded.

39. *Helichrysum expansifolium* stat. nov.

H. hookeri var. *expansifolium* Morris & Willis in Vict. Nat. **59**: 87 (1942);

H. alpinum Wakefield in Vict. Nat. **68**: 49 (1951) pro parte.

Holotype.—Cradle Mt., Tasmania, C. S. Sutton 2661, Feb. 1919 (MEL).

Paratype.—Cradle Mt. (valley), C. S. Sutton, Feb. 1919 (MEL).

Shrub with arachnoid-tomentose branches and erect habit like that of *H. hookeri*. *Leaves* linear but spreading at the base, acuminate-acute, 2–6 mm long, glabrous or with a few hairs above, the margins revolute and almost obscuring the very tomentose midrib below, erect or spreading but not appressed. *Capitula* subsessile in small clusters terminal to numerous short laterals towards the ends of the branches, 3.5–4.5 mm long, narrow or later narrow-turbinate. *Outer involucrel bracts* not numerous, woolly towards the base but scarious above and with slightly crumpled tips; *inner* with white erect or spreading laminae less than a third as long as the slender glabrous claw which is slightly thickened below. *Florets* 5–7. *Achenes* glabrous, the pappus bristles slightly thickened above.

The possibility of this taxon being the result of a cross between *H. hookeri* and some other species (possibly *H. ledifolium* (?)) has been considered and certainly the relationship with *H. hookeri* is very close. However, it is not only distinct but

has been collected in several localities so that if it is of hybrid origin then the inter-relationships throughout the subgenus must all be more complex than has been made obvious from this morphological study. In the lack of evidence one way or another it has been given specific status and only further investigation will reveal whether this has been unwise.

At the same time it cannot be agreed that it is so close as to warrant inclusion under *H. alpinum* Wakefield, which has been discussed above. It differs in the smaller leaves with their revolute margins and spreading bases, the habit of growth which is closer to that of *H. hookeri*, the smaller clusters of capitula which are subsessile and smaller in the overall dimensions, and the laminae of the bracts which are barely radiating instead of conspicuous as in *H. alpinum*.

Specimens, other than the types quoted above, which have been studied include:

TASMANIA: Cradle Mt., Mrs. Lindon, 1924 (KEW); Mt. Wellington 3500 ft, W. M. Curtis, Jan. 1952 (HO).

40. *Helichrysum hookeri* (Sond.) Druce in Rep. Bot. Exch. Cl. Manchr. 1916: 626 (1917); *Ozothamnus hookeri* Sond. in Linnaea 25: 509 (1853); *Helichrysum baccharoides* F. Muell. ex Benth., Fl. Austral. 3: 633 (1867); *Baccharis lepidophylla* DC., Prod. 5: 427 (1836); *Ozothamnus lepidophyllus* Hook. f. in Hook., Lond. J. Bot. 6: 120 (1847) non Steetz; *Helichrysum lepidophyllum* Tovey & Morris in Proc. Roy. Soc. Vict. 35: 195 (1923) non F. Muell. ex Benth. (1867).

Holotype.—"A glutinous shrub discovered growing on the black exposed summit of Mt. Wellington (elevation above the ocean 4100 feet) near Hobart Town, Van Diemen's Land, A. Cunningham Jany. 1819 (G-DC)" (= type of earliest combination).

Shrub to 2 m high, the branches at first tomentose but later glabrescent. *Leaves* closely appressed, erect, ovate-linear, viscid, the revolute margins almost concealing the tomentose midrib below, 1-2 mm long. *Capitula* subsessile in subglobose clusters at the ends of the upper branchlets, narrow, cylindrical but later narrow-turbinate and loose, 4 mm long. *Outer involucrel bracts* scarious-chartaceous, slightly viscid, not numerous; *inner* with white laminae not more than a quarter as long as the thin claw which is glabrous or bears a few hairs on the slightly thickened centre. *Florets* 2-4. *Achenes* minutely papillose, the pappus bristles thickened at their tips. (Fig. 11, A-A₄.)

The combination used in the "Flora Australiensis" is illegitimate and since Decandolle's epithet is pre-empted Druce was correct in applying that established by Sonder (see Morris and Willis in Vict. Nat. 59: 84 (1942)). Material and a photograph of the specimen used by Decandolle (for his *Baccharis? lepidophylla*) have been examined. The material is very immature, only young capitula being present, but there can be no doubt as to its identity.

The species is common at higher elevations in Tasmania and extends through Victoria to the Australian Alps though always associated with the higher levels and with places carrying winter snow. It is well represented in the herbaria of the



Fig. 11.—A–A₄, *H. hookeri* (Sond.) Druce (drawn from Burbidge 2901). B–B₄, *H. scutellifolium* (Hook. f.) Benth. (drawn from “Mt. Nelson, Rodway”). Lettering as in Fig. 2.

States most concerned and the following specimens are quoted in order to remove any possible ambiguity as to the author's opinion. It should be noted that the material present in the Kew Herbarium up until September 1954 has also been examined.

Specimens Studied.—AUSTRALIAN CAPITAL TERRITORY: Mt. Ginini, shrub 2·5 ft high, in peat swamp, *N. T. Burbidge* 2901, 12.iii.1949; Mt. Gingera, *C. W. E. Moore* 2439, 17.iii.1953. NEW SOUTH WALES: Mt. Kosciusko, *J. McLuckie and A. H. Petrie*, summer 1924–5; also *Brough*, January 1928. TASMANIA: Upper slopes of Mt. Barrow, *N. T. Burbidge* 3015, 9.i.1949; between Derwent Bridge and Bronte, *N. T. Burbidge* 3403, 27.i.1949; Lake Fenton, *O. Rodway* 116, 26.iv.1932; Mt. Wellington, *E. Rodway* 145, May 1931 (CANB).

41. *Helichrysum scutellifolium* (Hook. f.) Benth., Fl. Austral. 3: 633 (1867);
Ozothamnus scutellifolius Hook. f., Fl. Tasm. 1: 202.t.56 (1856).

Holotype.—Port Arthur, December 1845, *I. L. Burnett* (Gunn 1977) (KEW) (photograph No. 1612 at CANB).

Shrub to 1 m high, with tomentose stems. *Leaves* almost globular, 0·5–1 mm in diameter, closely reflexed-appressed, slightly viscid and sometimes with a very few long hairs, lower surface completely concealed but the margins revolute. *Capitula* sessile in small clusters at the ends of short laterals in the upper part of the stem, 3·5 mm long. *Outer involucral bracts* thinly woolly-villous, not numerous; *inner* with small white laminae which are more or less undulate and about a third or a quarter as long as the claw which is glabrous and thickened below, or sometimes with a few woolly hairs just below the junction of the laminae. *Florets* 14–18. *Achenes* minutely papillose-pubescent, pappus bristles very slightly thickened at the tip. (Fig. 11, *B–B₄*.)

At first glance this resembles *H. hookeri* but the curious reflexed leaves and the more numerous florets are distinctive. It appears to be restricted to Tasmania.

Specimens Studied.—TASMANIA: Tasmania, *Oldfield* (KEW); Dromedary, *F. H. Long* 306, 4.i.1931 (CANB); Bellerive, *W. M. Curtis*, Oct. 1943 (HO).

42. *Helichrysum lycopodioides* (Hook. f.) Benth., Fl. Austral. 3: 634 (1867);
Ozothamnus lycopodioides Hook. f. in Hook., Lond. J. Bot. 6: 119 (1847) and
Fl. Tasm. 1: 201.t.57 (1856).

Lectotype.—Nr. River Apsley, Great Swanport, *Burnett* (Gunn 1976) (KEW) (photograph No. 1614 at CANB).

Shrub to 1 m high, slightly viscid and apparently glabrous but under a strong lens numerous epidermal "globules" are visible. *Leaves* crowded, linear-oblong, obtuse, 5–7 mm long, the flat petiole-like bases adpressed to the stem and persistent, glabrous, slightly viscid, slightly concave above with the midrib in a shallow groove, margin flat, midrib slightly prominent towards the base below. *Capitula* in terminal globular clusters, sessile, surrounded by the uppermost leaves which almost form an involucre, 3·5–4 mm long. *Outer involucral bracts* stiff, viscid, with few long woolly hairs; *inner* with erect or slightly incurved purplish laminae which are sparsely villous on the back above the indurate claw, the margin of the laminae

usually decurrent on the claw. *Florets* about 20. *Achenes* papillose, the pappus bristles strongly barbellate and conspicuously thickened above.

Restricted to Tasmania and peculiar for the dark coloured inner involucre bracts. The growth of successive seasons arises from the uppermost axils of the preceding. This condition is also seen in *H. selaginoides* and to a lesser extent in some other species.

On the sheet at Kew there are six pieces of material arranged in two groups. Attached to the upper group are two labels which read: (1) "Sugar loaf Lit: Swan Port" and (2) "*Cassinia? Ozothamnus lycopodioides* n. sp. Nr. the Saddle between the Eastern Marshes and Swanport V.D.L.". Under the latter "Backhouse" has been written in by W. D. Hooker but the name on the label is in J. D. Hooker's hand. It is not altogether clear whether it refers to the upper group of material or the lower. On the label by the lower group is "1976 on River Apsley, Great Swanport, East Coast" also "1847" in Gunn's hand and Bentham has added his combination. On a further label pinned to the sheet but not specially directed to any specimen though presumed to apply to the lower group is the following note by Gunn: "1976 From near the River Apsley which runs into Great Swanport at the North End of Oyster Bay on the East Coast. I got the specimens at Hobart Town but they were collected by Mr. J. L. Burnett I believe though I cannot be certain who was the gatherer. My gardener also found it near the same locality." To this J. D. Hooker has added "*Ozothamnus lycopodioides* H.f.". It is believed that Gunn's labels refer to the flowering material, near to which Hooker's sketches are placed and that it should be selected as the lectotype.

EXCLUDED SPECIES

Helichrysum pholidotum (F. Muell.) F. Muell. ex Benth., Fl. Austral. 3: 634 (1867) based on *Ozothamnus pholidotus* F. Muell., Fragm. 2: 131 (1861) has been shown by J. M. Black (Trans. Roy. Soc. S. Aust. 43: 43 (1919)) to belong under *Humea*.

Helichrysum cunninghamii (DC.) Benth., Fl. Austral. 3: 629 (1867) should be returned to its original position under *Cassinia* (see Wakefield in Vict. Nat. 68: 70 (1951)).

Helichrysum angustum N. A. Wakefield (loc. cit., p. 49 (1951)) must be transferred to *Ixodia* R. Br.

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GENUS HELICHRYSUM SUBGENUS OZOTHAMNUS

HERBARIUM AUSTRALIENSE

DIVISION OF PLANT INDUSTRY—C.A.B.O.—CANBERRA.

No. 5382 Date 10. ii. 1956

Name *Helichrysum cordatum* D.C.

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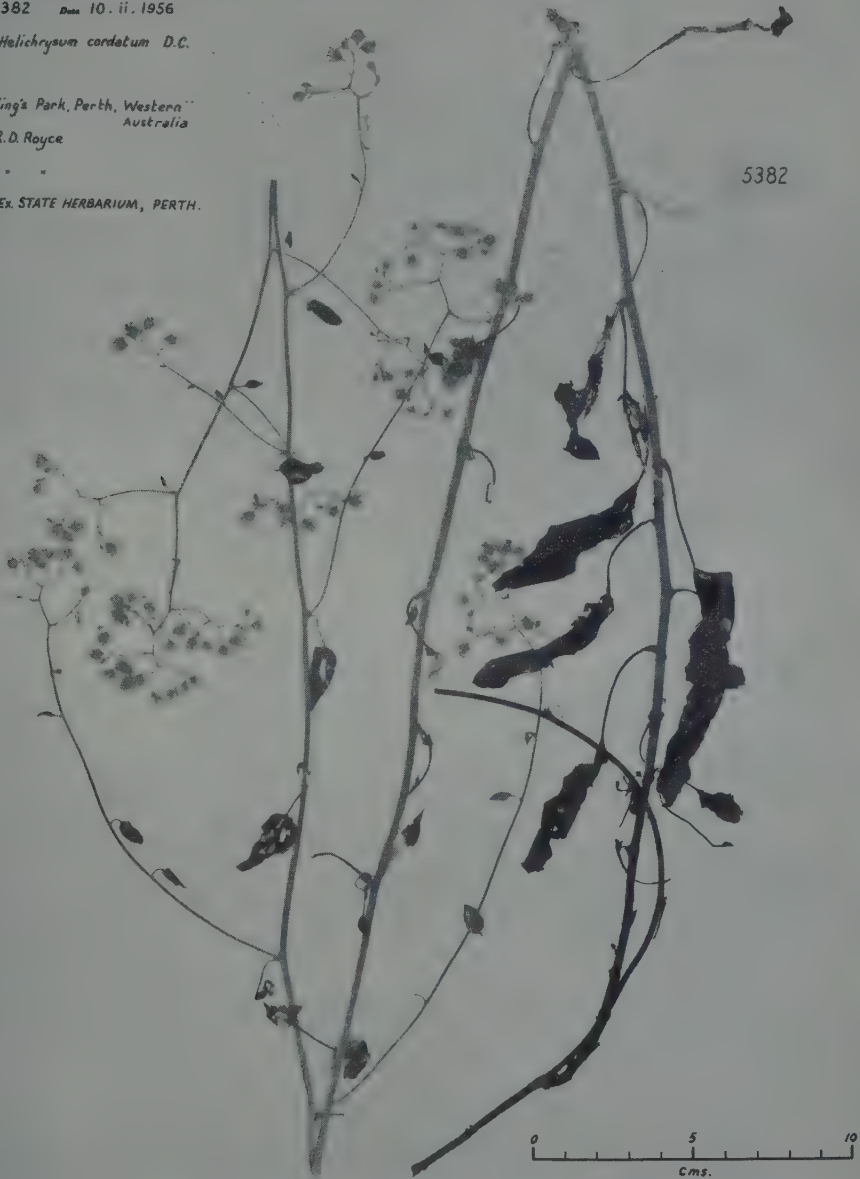
Loc. King's Park, Perth, Western
Australia

Coll. R. D. Royce

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Notes Ex. STATE HERBARIUM, PERTH.

1952



H. cordatum (photograph of Royce 5382).

FLORAL HISTOGENESIS IN THE MONOCOTYLEDONS

III. THE JUNCACEAE

By C. BARNARD*

[Manuscript received June 19, 1958]

Summary

An account is presented of floral histogenesis in *Luzula campestris* (L.) DC., *Juncus articulatus* L., and *Juncus vaginatus* R. Br.

The pattern of floral histogenesis in these species is similar to that in species of the Gramineae and Cyperaceae. Bracts, bracteoles, perianth members, and carpellary tissue arise by divisions of cells of the tunica. Periclinal divisions in the outer tunica layer (dermatogen) always occur in the initiation of these "foliar" type organs. The primordia of inflorescence branches, flower primordia, and stamens, on the other hand, arise as a result of cell division in the outer layers of the corpus and the inner layer of the tunica. Periclinal divisions in the dermatogen are not involved in the differentiation of these "cauline" type structures.

The morphological nature of the placentas in *Juncus* is discussed; they appear to arise independently of the carpellary tissue and after the manner of "cauline" structures.

The ovules in both *Luzula* and *Juncus* arise in similar fashion.

I. INTRODUCTION

Studies of floral histogenesis in the Gramineae and the Cyperaceae were reported in previous communications (Barnard 1957*a*, 1957*b*), and the general purpose of such studies was discussed and the relevant literature briefly reviewed. In the present paper an account is given of floral histogenesis in two genera of the family Juncaceae.

The Juncaceae have a world-wide distribution but are best developed in temperate, cold, and mountain regions. The family is composed of eight genera, five of which are restricted to cold or high montane habitats. One genus, *Prionium*, is restricted to South Africa. Of the other two genera, *Luzula*, with some 50 species, is found in temperate regions and more particularly in those of the northern hemisphere; whilst *Juncus*, with some 220 species, is cosmopolitan.

Hutchinson's (1934) view that this family is closely related to the Liliaceae and is composed of reduced forms derived from that stock is generally accepted. Hutchinson also believed that, because of their glumaceous bracts, reduced perianth, and general facies, the Juncaceae show a distinct tendency towards the evolution of higher and more reduced families such as Restionaceae and Cyperaceae.

For the present study *Luzula* and *Juncus* were examined. No account of floral histogenesis in these genera has previously been published. Stant (1952)

* Division of Plant Industry, C.S.I.R.O., Canberra.

and Catesson (1953) have described the organization of the vegetative apex in *Luzula*. Brenner (1922) dealt with the development of the embryo sac and endosperm of *Juncus* and *Luzula*. Payer (1857) alone has described floral organogeny in this family, and he used *Luzula campestris* (L.) DC. and *Juncus conglomeratus*. He remarks that, with the exception of the number of ovules, the structure of the flower is almost identical in the two plants.

Floral morphology is described in the present paper only to the extent that it is considered necessary for an understanding of floral histogenesis.

II. MATERIALS AND METHODS

Floral histogenesis was studied in detail in *Luzula campestris* (L.) DC. and *Juncus articulatus* L., and supplementary observations were made in *J. vaginatus* R. Br. and other species of the "genuini" group. Material of these species, covering all stages of floral development, was collected from naturally occurring plants in the environs of Canberra. *Luzula campestris* commenced to flower during July and continued to flower into October; *Juncus articulatus* commenced to flower during late October and continued to do so for several months also.

The techniques used for dissection and serial sectioning were the same as those previously described (Barnard 1957a, 1957b). The drawings likewise have been similarly prepared by photographing the subject and tracing an enlarged image from the negative.

III. MORPHOLOGY

(a) *Luzula campestris* (L.) DC.

The primary branches of the inflorescence of *Luzula campestris* form an irregular umbel. Secondary branches, each carrying from two to ten flowers, are clustered to form ovoid or globular heads at the ends of the umbel branches. The whole inflorescence is subtended by a large leaf-like bract; and each primary and secondary branch by a glumaceous bract. There is also an empty hyaline bract at the base of each branch on its adaxial side. The elongation of the internode between the empty bract and the bract which subtends the first secondary branch is greatest in the lower primary branches. The primary branches are therefore of unequal length, the lower being the longer. Each secondary (or sometimes tertiary) branch bears from one to nine lateral flowers and a terminal flower, each flower being subtended by a small bract. The flower pedicel bears two hyaline and lobed bracteoles, the lower being adaxial and the upper abaxial in position. Both bracteoles almost completely encircle the pedicel. The lateral flower primordia develop in acropetal succession. The terminal flower is formed directly from the growing point of a branch, and, during its early stages of development, is slightly more advanced than the upper lateral flower primordia.

The flower has six free, imbricating, and glumaceous perianth segments, six erect stamens, a unilocular ovary with three basal erect and anatropous ovules, and three linear, but slightly twisted, stigmas. The six perianth segments arise in spiral sequence (Plate 1, Fig. 1) but early in their development become defined as two whorls of three segments each. The two first-formed segments are in

anterolateral positions and the third segment is posterior (abaxial) in position. The stamens arise in the axils of the six perianth segment primordia and are also arranged in two whorls of three. The ovary is first seen as a ridge of tissue which encircles the growing point of the flower primordium. Upon this ridge, three protuberances or carpel lobes appear, and the first of these lobes is on the same side of the floral axis as, and opposite to, the first perianth segment. The ridge grows upwards to form a tube carrying the three carpel tips. The carpel tips also grow so that the ovary soon appears as a tubular structure with a deeply trilobed tip. The three ovules appear as papillae inside the base of the tube and opposite the carpel tips. The carpel tips develop into the stigmas.

(b) *Juncus articulatus* L. and *J. vaginatus* R. Br.

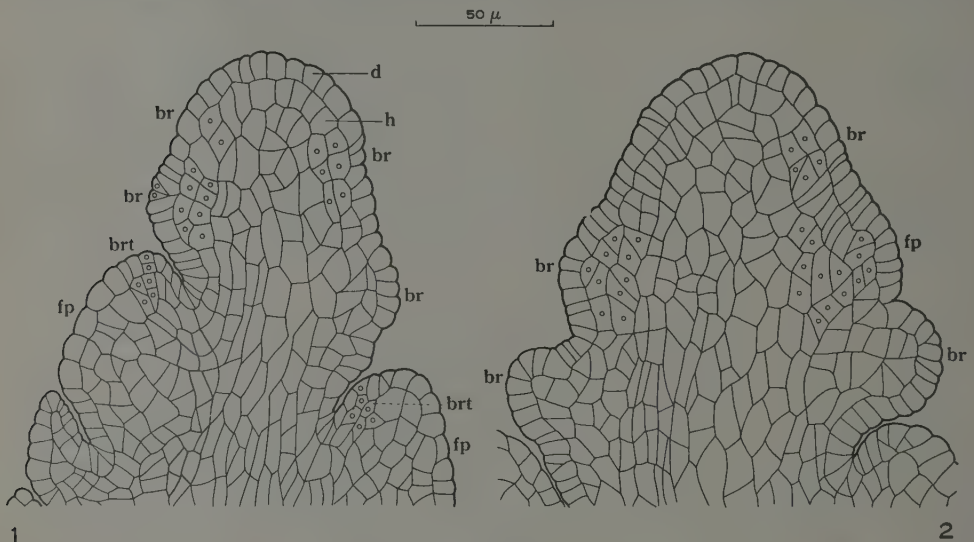
The inflorescence of *Juncus articulatus* is a cymose panicle, with branches, usually of the third order, bearing clusters of flowers in more or less globular heads. Each branch is subtended by a narrow pointed bract and bears a smaller truncated and empty bract on its adaxial side. Each flower occurs in the axil of a small bract. The first-formed flower primordia on a branch develop more rapidly than the succeeding ones. By the time the third or fourth flower primordium is being initiated the first is usually already overtopping the growing point of the branch. From two to ten flower primordia reach maturity to form the flower cluster; the apex and the youngest flower primordia abort.

No bracteoles are developed on the pedicel of the flower. In the absence of bracteoles and of terminal flowers, *J. articulatus* differs not only from *Luzula* spp., but also from a number of other species of *Juncus*. In *Juncus bufonius* L., and *J. homalocaulis* F. Muell., as well as *J. polyanthemos* R. Br., *J. vaginatus* R. Br., *J. filicaulis* Buchen., and *J. pallidus* R. Br. of the "genuini" group, bracteoles are formed and branches terminate in a flower. In *J. polyanthemos*, a lateral branch is formed immediately below the terminal flower and this lateral aborts. Thus the flowers appear singly at the ends of branches and just below each one is a small hyaline bract which subtended the aborted lateral. These bracts may appear very like a third bracteole to the flower. In *J. vaginatus*, the flowers are usually in pairs (at the ends of branches of a cymose panicle). Both flowers are terminal; one terminates a short lateral from the branch upon which the other is terminal. A lateral of the second order aborts. Thus the flower which terminates the main branch has two bracteoles; the flower which terminates the lateral appears to have three bracteoles, but one of these is the bract which subtends the aborted lateral. *J. filicaulis* and *J. pallidus* have a comparable morphology. *J. capitatus* Buchen., like *J. articulatus*, has no bracteoles.

The flower has six free, imbricate perianth segments, six erect stamens, and a trilocular ovary, with parietal placentas and numerous ovules. Its three linear stigmas are markedly twisted or corkscrewed. At maturity, the perianth segments are glumaceous and are arranged in two whorls of three. Though similar at maturity, the members of the inner whorl differ from those of the outer whorl during their early development. The three outer segments arise in sequence and there is an appreciable difference in size between the successive segments. Quite early in their

development they assume the lanceolate shape which they have when fully grown but only gradually become uniform in size. The three inner perianth segments, on the other hand, arise almost simultaneously and are initially broadly ovate in shape. As development proceeds they gradually assume the same shape and size as the outer members.

The stamens arise in two whorls almost simultaneously, those of the outer whorl being axillary to the outer perianth segments and those of the inner whorl axillary to the inner perianth segments. When the stamen initials are showing as papillae upon the flower primordium, a carpellary ridge is formed, which encircles its growing point and gives the apex a flattened appearance. Upon the ridge, in positions opposite the three outer perianth segments and stamens, three small protuberances (carpel tips) develop. Up to this stage development has been similar



Figs. 1, 2.—*Luzula campestris*. Longitudinal sections of the apex of inflorescence branches showing organization of tissues into dermatogen (*d*), hypodermis (*h*), and corpus, and the origin of bracts (*br*), bracteoles (*brt*), and flower primordia (*fp*).

to that of *Luzula*; it now proceeds differently. Three papillae develop on the inner side of the ridge and confluent with it at points midway between the carpel tips. These papillae, which form the placentas, extend inwards to the centre of the growing point of the flower primordium. The carpellary ridge grows upwards to form a tube carrying the three carpel tips at its apex, and the three placentas along its inner surface. The tissue at the top of the tube later becomes confluent to close the wall of the ovary, and the three stigmas develop from the carpel tips. The ovules are formed in two rows along each placenta.

IV. HISTOGENESIS

(a) *Luzula campestris* (*L.*) DC.

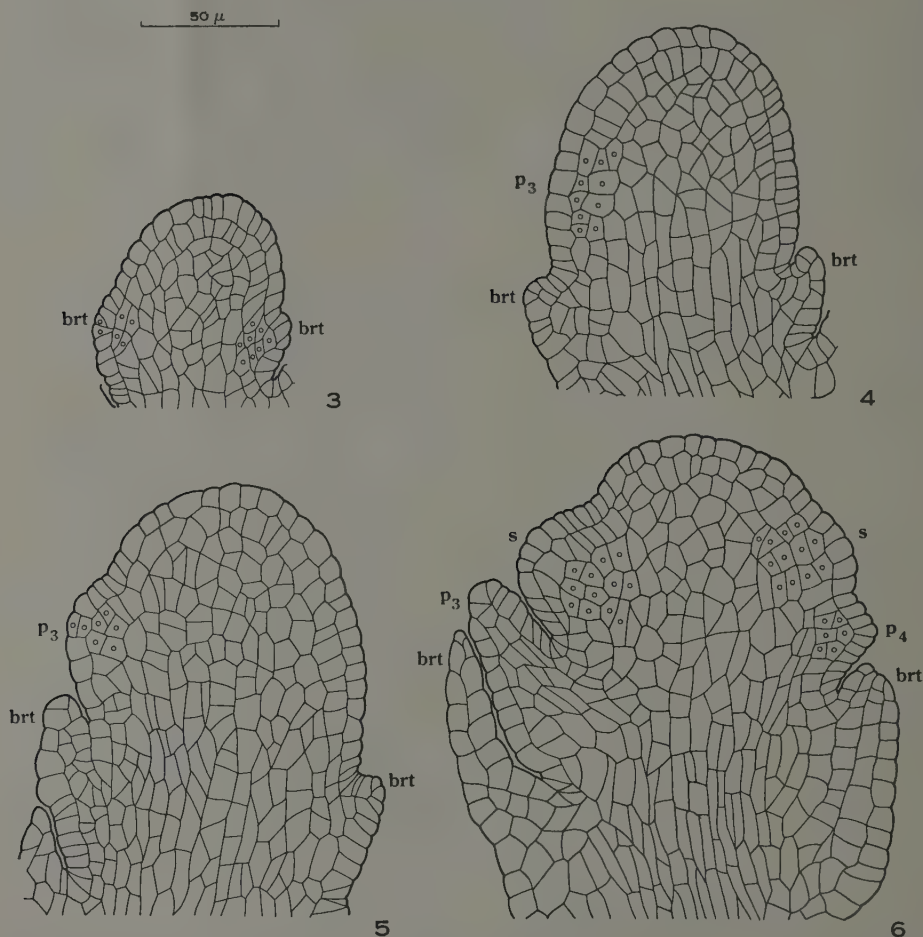
(i) *Apex*.—The apex of each branch of the inflorescence has a two-layered tunica surrounding a central corpus (Figs. 1, 2). Cells of the outer tunica layer, or

dermatogen, divide only by anticlinal walls, except where a bract primordium is being initiated. The inner tunica layer, or hypodermis, is clearly defined, its cells tending to be slightly more elongated in the radial direction than those of the dermatogen. Cells of the hypodermis divide only by anticlinal walls except where a bract primordium, a lateral branch primordium, or a flower primordium is formed. Cells of both the dermatogen and hypodermis have large nuclei and dense cytoplasm which stains deeply. These characteristics and the regular disposition of their cells distinguish the tunica from the corpus. A small group of cells at the tip of the corpus also has large nuclei and dense cytoplasm; these cells divide in various planes but tend to become disposed in longitudinal files. The cells quickly become vacuolated and, back from the tip of the corpus, cells in this zone stain less deeply than those of the tunica. There is considerable variation in the size of the apices of different branches (cf. Figs. 1, 2).

(ii) *Origin of Bracts*.—The bracts which subtend branches of the inflorescence and those which subtend flower primordia arise in the same way. On one side of the apex periclinal divisions occur in a horizontal plate of hypodermal cells some five or six cells below what may be termed the apical hypodermal cell (Fig. 1). Anticlinal divisions in dermatogen and hypodermal cells above this plate continue so that the distance between the plate and the tip of the apex increases. At the same time, hypodermal cells just above and below those which underwent the first periclinal division also divide periclinally (Figs. 1, 2). A horizontal plate with a vertical depth of some four or five cells is thus formed by hypodermal cells which have divided periclinally near the centre of initiation and with inclined walls at the peripheries of the area of differentiation. At this stage the bract primordium is visible as a small crescentic ridge behind the tip of the apex. The ridge increases in size by the continued division of derivatives of the original hypodermal cells (Fig. 2). Periclinal and inclined divisions then occur in the dermatogen at the centre of the area of initiation (Fig. 1) and further increase in size of the young bract primordium is effected largely by divisions of this kind. The primordium ridge thus expands as a result of marginal meristematic activity. Cells of the outer layer of the corpus (subhypodermis), below the centre of the area of insertion, also divide once or twice by periclinal walls. These cells become vertically elongated and form part of the provascular trace leading to the bract. The corpus makes no contribution to the tissue of the bract primordium.

(iii) *Origin of Branch and Flower Primordia*.—Branch and flower primordia arise in a different way from the bracts. Hypodermal cells at the site of initiation become slightly elongated in the radial direction and cells in the subhypodermis divide periclinally. At this stage, a flower or branch primordium may be detected as a very small bulge just above the centre of insertion of the bract primordium (Fig. 2). Periclinal divisions occur in the elongated hypodermal cells at and near the centre of the differentiating area and inclined divisions in those at its periphery. Where hypodermal cells have divided periclinally the outer cell becomes part of the hypodermis of the flower primordium, the inner one part of the corpus. Further periclinal divisions occur in subhypodermal cells. The dermatogen cells continue to divide only by anticlinal walls. Thus in the young flower or branch primordium the dermatogen is continuous with, and derived solely from, the dermatogen of the

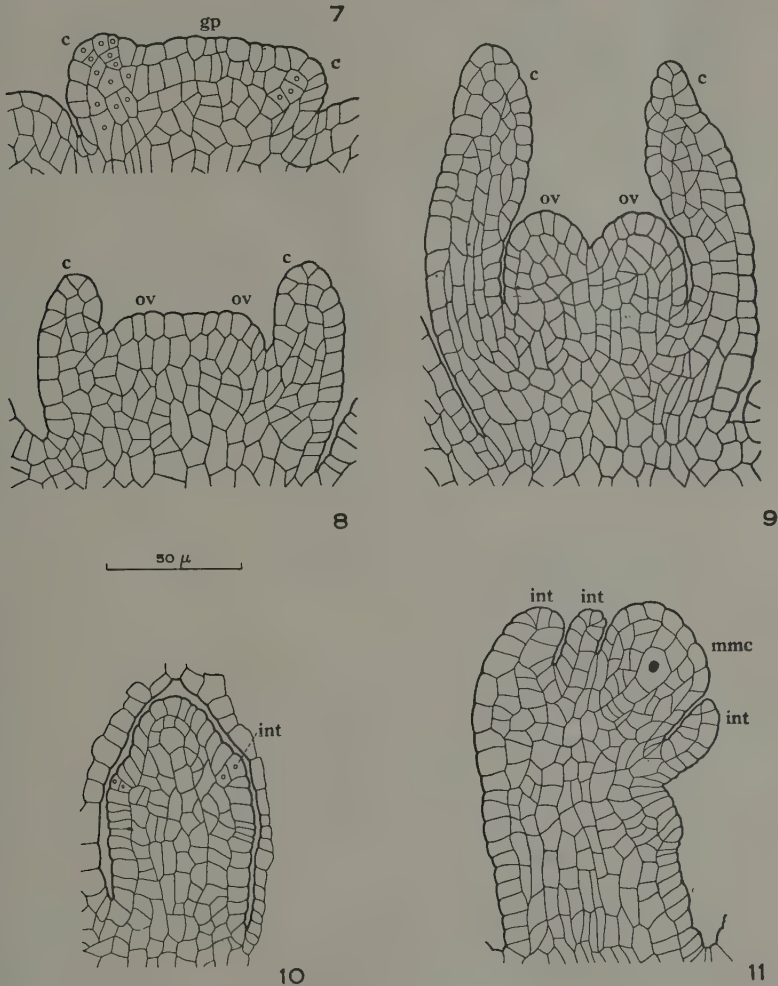
axis from which it arises, and the hypodermis is derived solely from the hypodermis of the axis. The corpus of the flower primordium is derived predominantly from the corpus, and to a lesser extent from the hypodermis, of the axis. In the young flower primordium the cells of the corpus are densely cytoplasmic and stain deeply but become vacuolated as the primordium grows.



Figs. 3-6.—*Luzula campestris*. Longitudinal sections of flower primordia. Fig. 3.—Very young primordium showing origin of bracteoles (*brt*). Figs. 4, 5.—Slightly older primordia showing origin of third perianth member (p_3). Fig. 6.—An older flower primordium showing the third and fourth perianth members (p_3 and p_4) and the origin of the stamens (*s*) axillary to these structures.

(iv) *Differentiation of Flower Primordia*.—The adaxial bracteole arises at a very early stage in the development of the young flower primordium. Periclinal divisions occur in hypodermal cells and then in dermatogen cells (Fig. 3). The abaxial bracteole arises almost simultaneously in similar fashion. Further development of the bracteoles and the flower primordium is illustrated in Figures 4-6 and Plate 1, Figure 2. Growth of the bracteoles results primarily from the division of

a series of triangular dermatogen cells which, by cutting off cells with inclined walls, behave like true apical cells. Derivatives of hypodermal cells of the flower primordium contribute little to the tissue of the bracteole. The mode of origin of



Figs. 7-11.—*Luzula campestris*. Fig. 7.—Longitudinal section of apex of flower primordium showing growing point (*gp*) and origin of carpellary ridge (*c*). Fig. 8.—Shows further development of the carpellary ridge and the origin of the ovules (*ov*). Fig. 9.—Shows further development of the ovules. Fig. 10.—Longitudinal section of ovule in which the outer integument (*int*) is arising. Fig. 11.—Longitudinal section of ovule with two integuments and megaspore mother cell (*mmc*).

the bracteoles is thus very like that of the bracts, but here marginal growth from dermatogen cells occurs earlier.

The perianth segments arise in a manner similar to that of the bracts. Periclinal and inclined divisions in hypodermal cells initiate their differentiation. (Figs. 4-6 and Plate 1, Fig. 2.) Subsequently periclinal and inclined divisions occur

in the dermatogen. Growth in the young perianth segment is the result of division of derivatives of both hypodermal and dermatogen cells.

The stamens, on the other hand, originate in a manner comparable with that of the flower primordia; periclinal and inclined divisions occur in both hypodermal and subhypodermal cells but not in the dermatogen (Fig. 6 and Plate 1, Fig. 2). The outer cells resulting from the divisions of the hypodermis become the hypodermis of the stamen primordium.

The carpellary ridge is initiated by periclinal divisions in the hypodermis, followed immediately by similar divisions in the dermatogen (Fig. 7 and Plate 1, Fig. 4). Early development of both the carpellary ridge and the carpel tips is mainly the result of divisions of the derivatives of the dermatogen cells.

The whole of the apex of the flower primordium is involved in the formation of the ovules. Elongation of hypodermal cells (Fig. 8) is followed by periclinal division of these and of underlying subhypodermal cells. As a result, three small papillae are developed. No periclinal divisions occur in the dermatogen. The origin of the ovule is therefore similar to that of the cauline structures (branch, flower, and stamen primordia). The organization of its cells is shown in Figure 9 and Plate 1, Figure 6. The bulk of the tissue of the integuments of the ovule is derived by divisions from the dermatogen cells though divisions in the hypodermis may also take place (Fig. 10). The outer integument partially encircles, and the inner one completely encircles, the ovule (Fig. 11).

(b) *Juncus*

Histogenesis in *Juncus* follows the same pattern as in *Luzula*. Apices of the branches of the inflorescence have each a two-layered tunica and a corpus (Figs. 12, 13, and Plate 1, Fig. 3). Bracts arise by division, first in hypodermal cells, then in dermatogen cells. The branch and flower primordia, on the other hand, arise primarily as the result of periclinal and inclined division of subhypodermal cells.

A lateral branch of *Juncus vaginatus* is shown in Plate 1, Figure 3. The apex of this branch will form a flower. At its base there is an adaxial and an abaxial bract. The former is empty but in the axil of the latter a secondary lateral branch primordium (*bp*) has been initiated. The first bracteole (*brt*) is developing on the adaxial side (left) of the main lateral and the origin of the second bracteole is apparent in a periclinal division of a hypodermal cell on the right. Further development of a similar lateral branch is illustrated in Figure 13. The two bracteoles are here well formed and the apex of the branch has broadened and two perianth segments are arising upon it. Two bracts are developing at the base of the lateral branch primordium. The apex of this secondary branch will also differentiate as a flower primordium and the hypodermal divisions initiating its first bracteole are already present. A tertiary lateral (*bp*) is arising in divisions in subhypodermal cells just above the abaxial bract in the secondary lateral. This tertiary lateral primordium will abort.

The apex of a branch of *Juncus articulatus* is shown in Figure 12. A bract is arising on the right side of the apex whilst a small flower primordium can be seen

on the left. There is a larger flower primordium on the right. The next oldest flower primordium on this branch was comparatively large, had well-developed carpels, and completely overtopped the growing point. In this species, the apex

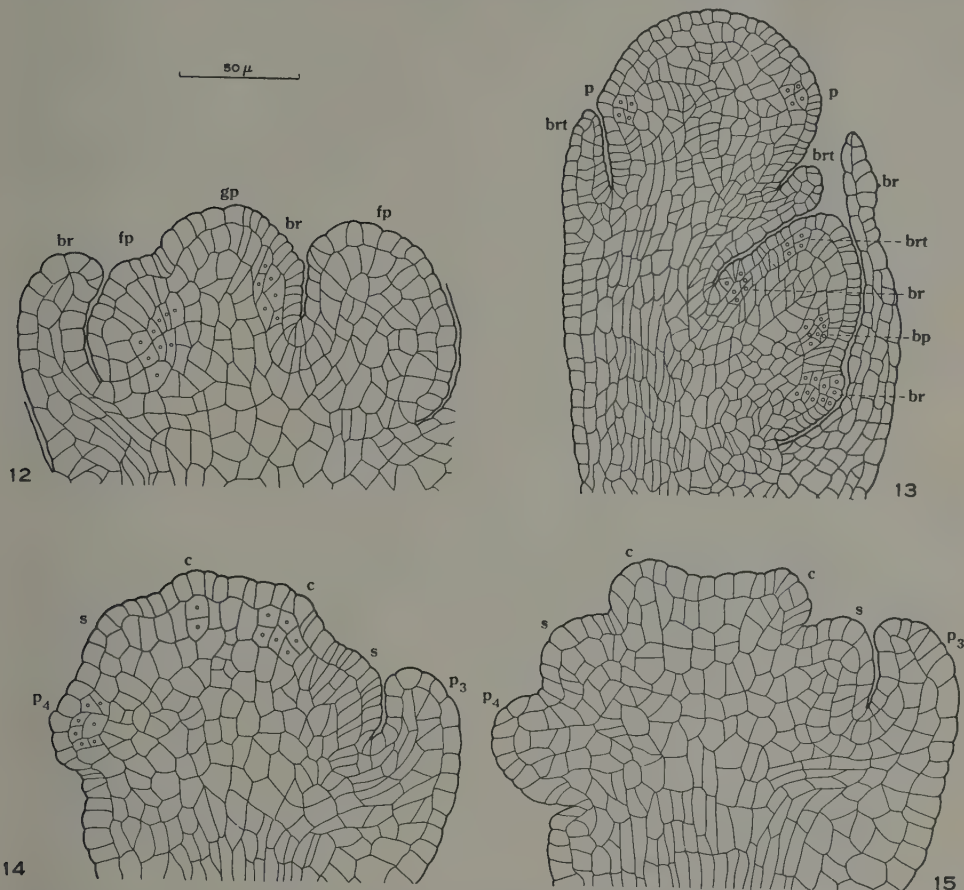
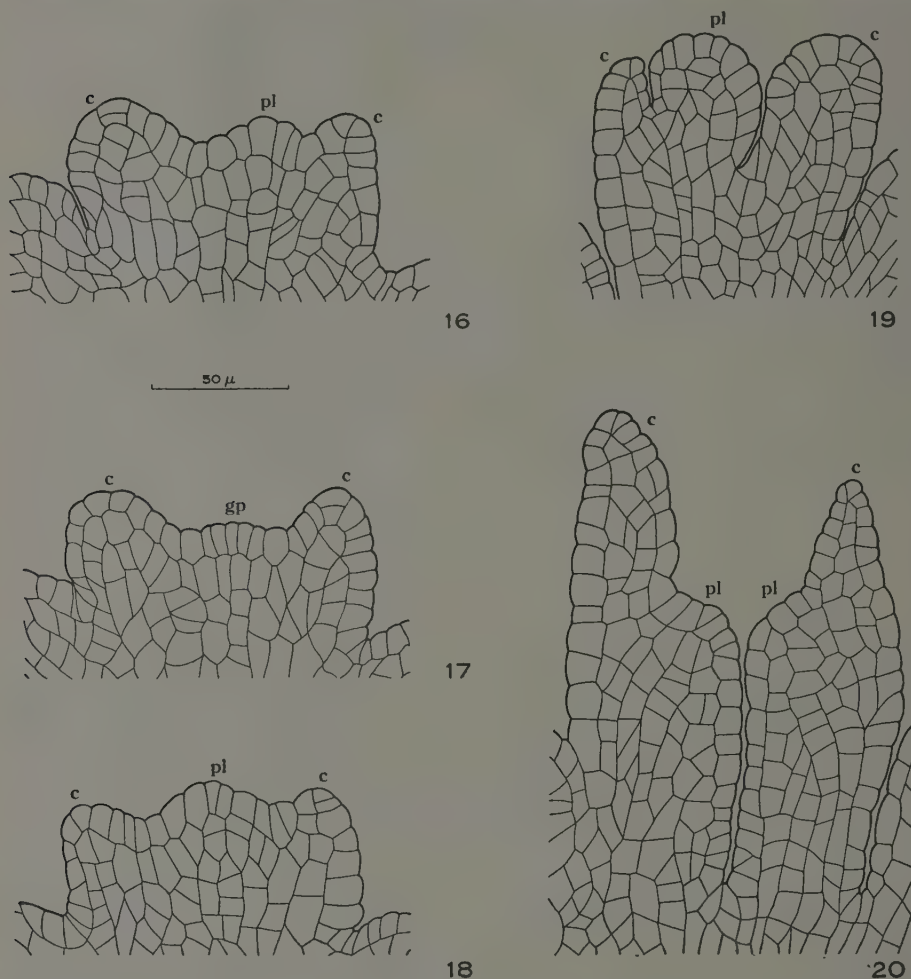


Fig. 12.—Longitudinal section of apex of inflorescence branch of *Juncus articulatus* showing growing point (gp), origin of bract (br), and flower primordium (fp). Fig. 13.—Longitudinal section of apex of inflorescence branch of *Juncus vaginatus*. The apex has become a flower primordium with two bracteoles (brt) and on it two perianth members (p) are being initiated. In the axil of the bract (br) upon this branch a lateral branch primordium is developed. Upon this branch primordium an adaxial and abaxial bract (br) are differentiating and a further branch primordium (bp) is forming in the axil of the abaxial bract initial. The apex of this lateral primordium will form a flower and the divisions in the hypodermis shown at brt are the first indication of the formation of its adaxial bracteole. Figs. 14, 15.—Longitudinal sections of flower primordia of *Juncus articulatus* showing third and fourth perianth members and the origin of the stamens (s) and carpellary ridge (c).

and last-formed flower primordia abort. No bracteoles are formed and the first structures developed from the flower primordia are the perianth members. The first two perianth members arise in lateral positions on the primordium. The third perianth segment is developed in an abaxial position and so is cut medianly

in radial longitudinal section of a branchlet. The modes of origin of the perianth members, stamens, and carpels are shown in Figures 14 and 15. In both figures, the third perianth segment is on the right and the fourth on the left; and in the axil



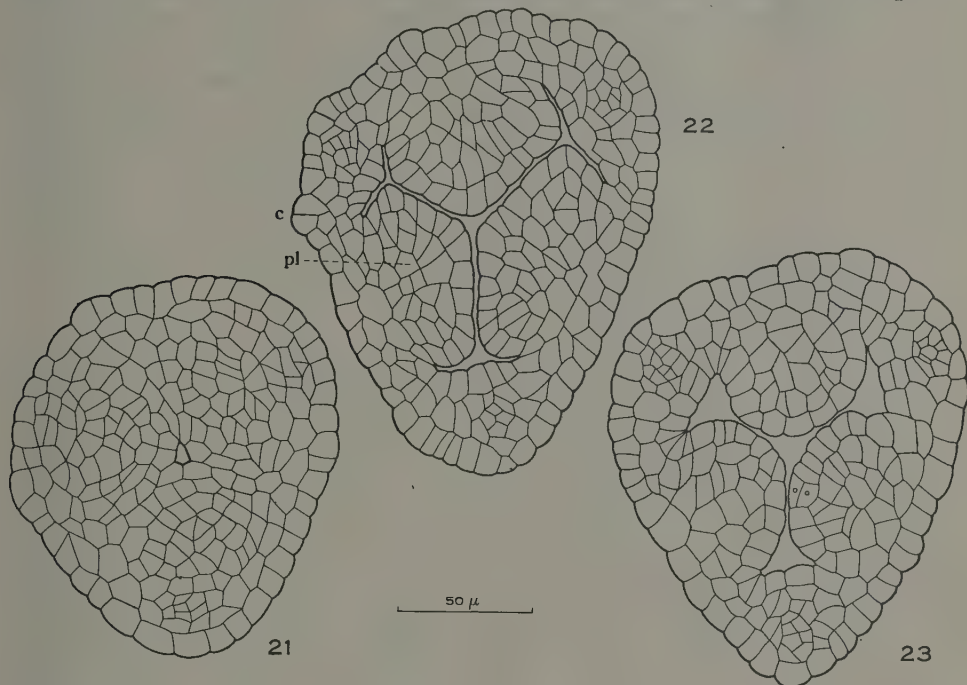
Figs. 16-20.—*Juncus articulatus*. Longitudinal sections through apices of flower primordia illustrating development of carpellary ridge and placentas. Figs. 16-18 are serial sections through the same apex showing the origin of the carpellary ridge (*c*) and a placenta (*pl*). Fig. 17, which illustrates a section between Figs. 16 and 18, shows the growing point (*gp*). Fig. 19 is a section median for one young placenta (*pl*). Fig. 20 is a section nearly median for two placentas. At this stage fusion of the placentas to the carpellary tissue is complete.

of each a stamen primordium is developing. In Figure 15 the carpels are arising. The histogenesis of these flower parts is the same as in *Luzula*.

The placentas appear to arise independently of the carpellary ridge and in a manner very like that in which the ovules arise in *Luzula*. Figures 16, 17, and 18 are sections one, three, and six of a serial ribbon (cut longitudinally at 7 μ)

through the apex of a flower primordium. In the first section (Fig. 16), one centre of placental initiation is apparent and in the sixth section (Fig. 18) another is developing. Elongation of hypodermal cells, subsequent periclinal division of these cells, and the division of subhypodermal cells characterize this initiation. Initiation seems to be independent of the carpellary ridge and periclinal divisions of the dermatogen are not involved.

The centres of placental initiation at this stage are axillary to the carpel tips and this placement is apparent in the transverse section shown in Figure 21. However, each centre of initiation divides into two halves and as development



Figs. 21-23.—*Juncus articulatus*. Transverse sections of a very young ovary. Fig. 21 is of a section from the base of the ovary. Fig. 22 is of a section half way up the ovary, and Fig. 23 of a section near the tips of the placentas (*pl*). The origin of placental tissue from a position opposite the mid points of the carpels is seen in Fig. 21; the later intercarpellary position of the placentas and their bilobed nature is shown in Fig. 22; whilst in Fig. 23 a periclinal division in the dermatogen of one of the placentas is depicted.

proceeds each half moves to a lateral position. Adjoining halves fuse in the intercarpel tip positions to form the bilobed placenta primordia (Fig. 22). In Plate 1, Figure 5, and in Figures 19 and 20 the placenta primordia are illustrated in longitudinal section. In their mode of growth, as well as in their manner of origin, they appear to be cauline structures fused to the ring of carpellary tissue.

In transverse section, however, periclinal divisions have been observed to occur in dermatogen cells in the upper portion of well-developed placenta primordia. Such a division is clearly shown in Figure 23, which is of a transverse section through the young ovary near the tips of the placentas.

V. DISCUSSION

The organization of the apices of the inflorescence branches of both *Luzula* and *Juncus* into a two-layered tunica and a central corpus is similar to that described for the Gramineae and the Cyperaceae (Barnard 1957*a*, 1957*b*). The mode of origin of the bracts and of the flower primordia is also similar in all species of these families. The bracts (or glumes) arise as a result of periclinal divisions of hypodermal cells, followed by the similar divisions of dermatogen cells. Whilst in the Gramineae, divisions in the dermatogen cells occur immediately following the first divisions in the hypodermis, in the Cyperaceae and Juncaceae periclinal division of dermatogen cells is delayed until the young glume or bract has become a well-developed crescentic ridge around the apex. Thereafter, periclinal division of dermatogen cells is frequent and they contribute largely to the growth of the young primordium. Neither Stant (1952) nor Catesson (1953) observed periclinal divisions in the dermatogen during the initiation of foliage leaves in *Luzula* spp. The flower primordium, on the other hand, is initiated by periclinal and inclined divisions in hypodermal and subhypodermal cells. No periclinal divisions occur in the dermatogen. A hypodermal layer is formed and the organization of the young flower primordium becomes, with dermatogen, hypodermis, and corpus, comparable with that of a branch apex.

The apices of the inflorescence branches of species in the three families Gramineae, Cyperaceae, and Juncaceae differ in the location of the floral bracts and flower primordia upon the axis rather than in the histogenesis of these organs. The flower primordia, each with its subtending bract, may be arranged distichously, developed in various spiral sequences, or be terminal on the inflorescence branch. In the Juncaceae, the ultimate branches of the inflorescence may: (i) develop lateral flower primordia in acropetal succession and a terminal flower primordium, and all primordia reach maturity (*Luzula*); or (ii) develop lateral flower primordia in acropetal succession, the apex of the branch, however, together with the youngest lateral flower primordia, aborting (*Juncus articulatus*); or (iii) develop terminal flowers only, lateral branches aborting (*Juncus* spp. of the *genuini* group). Whilst floral structure is uniform in *Juncus* considerable differences exist in the mode of growth of the inflorescence. From the present studies, it is not possible to draw any conclusions as to the evolutionary development of the inflorescence in this family. Both types (ii) and (iii) above could have been derived from type (i). It is worthy of note, however, that in such a group of plants as the Juncaceae, where floral structure is so uniform, study of inflorescence form and development should assist in establishing possible evolutionary trends within the group. Bews (1929) has shown in the Gramineae, where floral structure is also remarkably uniform and distinctive, that the evolutionary trend in the inflorescence seems to have been from many-flowered to few-flowered spikelets and that reduction has occurred both basipetally and acropetally. In general, however, the study of the evolution of inflorescence forms seems to have received less attention than it deserves.

The presence of bracteoles in some species of *Juncus* and their absence in others is a notable feature. The mode of origin of the perianth members is similar to that of the bracts and bracteoles, to the glumes, palea, and lodicules in the

Gramineae, and to the bracts and perianth members in the Cyperaceae. Likewise the "foliar" type origin of the carpellary structures is similar in all species of these families. The stamens, on the other hand, arise in the "cauline" manner as in the Gramineae and Cyperaceae.

The origin of the placentas in *Juncus* is of particular interest because the morphological nature of the placentas in the angiospermic flower has long been the subject of controversy. Douglas (1944) and more recently Puri (1952) have reviewed the problem. According to Puri the majority of workers agree that the placentas are carpellary structures in all flowers, but some think that they are receptacular or axial in origin in some or all angiosperms. The "classical" viewpoint, that the placentas are carpellary structures, interprets them as modified carpel margins. Marginal placentation is considered the most primitive form. Parietal placentation is considered more primitive than axile, and free central and basal placentation derived from either parietal or axile types. Van Tieghem, Eames, Troll, Douglas, Joshi, Puri, and many other workers have expounded this viewpoint. Schleiden, Payer, McLean Thompson, Hagerup, and others, however, have supported the view that the placentas are axial (or cauline) structures, although they differed in details of their interpretation. Payer (1857) believed that the margins of the carpellary leaf were overlain with outgrowths of the branched floral axis and the ovules were therefore borne on axial tissue. Hagerup, according to Puri (1952), concluded that the carpels merely form an involucre of coalescing and barren leaves around the floral axis which bears ovules. In ovules with parietal placentation, he believed, the ovuliferous floral axis has split into several distinct parts which become attached to the wall of the ovary at the junction of the carpellary margins. Proponents of the axial nature of placentas usually, though not necessarily, interpret free central or basal placentation as primitive. The histogenesis of placental development was not examined by any of the proponents of either school as a means of providing evidence for their conclusions.

In *Juncus* spp. the histogenesis of the placentas rather supports the view that they are axial structures fused to the encircling carpellary tissue. The mode of origin of the placentas is quite different from that of the carpellary tissue; they arise as cauline structures. The carpellary tissue, on the other hand, arises by divisions in the hypodermis and dermatogen in the "foliar" manner. The beginning of placental differentiation axillary to the carpel tips with a subsequent division of this centre of initiation into two halves which take up lateral positions would seem to support Hagerup's hypothesis rather than the classical theory. If the bilobed placentas do represent the fused modified margins of adjoining carpels, it is difficult to see why they do not, from the beginning, occupy marginal carpellary positions.

One observation makes the conclusion that the placentas are axial structures uncertain: the occurrence of periclinal divisions in the dermatogen near the extremities of well-developed placentas. It is possible, however, that these divisions are associated with carpellary tissue. The fusion which occurs between placental and carpellary tissue during development could result in an admixture of cells from the two structures particularly towards the extremities of the placentas.

The periclinal divisions observed in the dermatogen of the placentas could be so interpreted.

In *Luzula*, the ovules arise directly from the growing point of the flower primordium as cauline structures, and, in their histogenesis, show no connexion with the carpellary tissue. Thus there is no histogenic evidence to support the view that their basal placement is a condition derived from a former parietal one. In *Juncus* the ovules arise as cauline structures from the placentas. The integuments in both genera arise by division of dermatogen and hypodermal cells of the ovule and are thus "foliar" in origin.

VI. REFERENCES

- BARNARD, C. (1957a).—Histogenesis in the monocotyledons. I. The Gramineae. *Aust. J. Bot.* **5**: 1–20.
- BARNARD, C. (1957b).—Histogenesis in the monocotyledons. II. The Cyperaceae. *Aust. J. Bot.* **5**: 115–28.
- BEWS, J. W. (1929).—"The World's Grasses: their Differentiation, Distribution, Economics, and Ecology." (Longmans, Green and Co.: London.)
- BRENNER, W. (1922).—Zur Kenntnis der Blütenentwicklung einiger Juncaceen. *Acta Soc. Sci. Fennica* **50**: 1–27. (*Bot. Abstr.* **15**: abstr. 8122 (1926).)
- CATESSON, ANNE-MARIE (1953).—Structure, Evolution et Fonctionnement du point végétatif Monocotylédone; *Luzula pedemontana* Boiss. et Reut. (Joncacées). *Ann. Sci. Nat. (Bot.)* (11)**14**: 253–91.
- DOUGLAS, GERTRUDE E. (1944).—The inferior ovary. *Bot. Rev.* **10**: 125–86.
- HUTCHINSON, J. (1934).—"The Families of Flowering Plants. II. Monocotyledons." (Macmillan & Co.: London.)
- PAYER, J. B. (1857).—"Traite d'Organogenie Comparée de la Fleur." (Masson: Paris.)
- PURI, V. (1952).—Placentation in angiosperms. *Bot. Rev.* **18**: 603–51.
- STANT, M. Y. (1952).—The shoot apex of some monocotyledons. I. Structural development. *Ann. Bot., Lond. (N.S.)* **16**: 115–28.

EXPLANATION OF PLATE I

Photomicrographs of *Luzula* and *Juncus*

br, Bract; *bri*, bracteole; *p*₁–*p*₄, perianth members; *bp*, branch primordium; *s*, stamen; *c*, carpel; *cr*, carpellary ridge; *pl*, placenta; *ov*, ovule.

Fig. 1.—*Luzula campestris*, flower primordium with carpellary ridge developing. ×84.

Fig. 2.—*L. campestris*, longitudinal section of flower primordium showing origin of stamens. ×326.

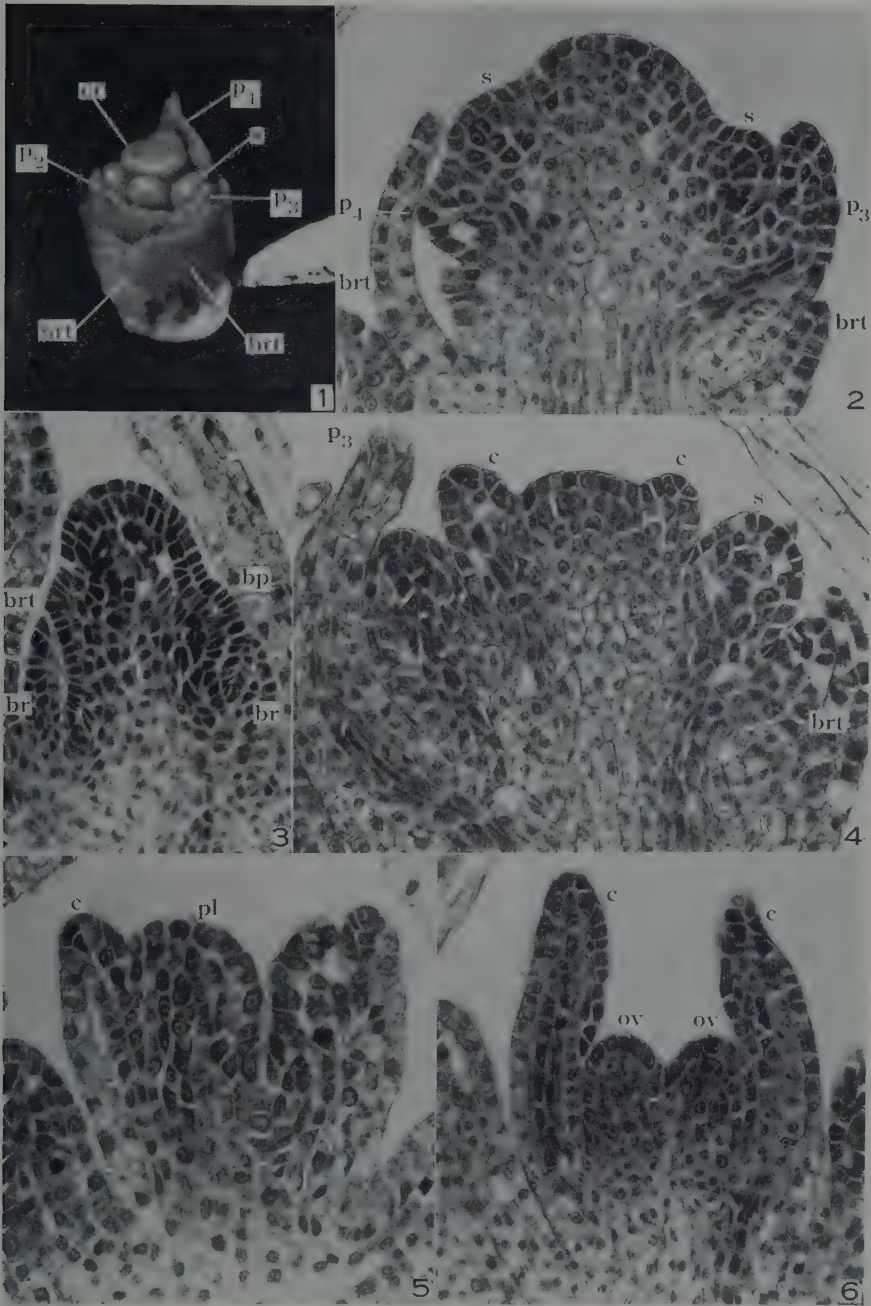
Fig. 3.—*Juncus vaginatus*, longitudinal section of lateral branch, the apex of which will form a flower primordium. A secondary lateral branch primordium is developed at *bp*. ×326.

Fig. 4.—*Luzula campestris*, longitudinal section of flower primordium in which the carpels (carpellary ridge) are developing. ×326.

Fig. 5.—*Juncus articulatus*, longitudinal section of apex of flower primordium showing early development of a placenta. ×326.

Fig. 6.—*Luzula campestris*, longitudinal section of apex of flower primordium showing young ovules. ×326.

FLORAL HISTOGENESIS IN THE MONOCOTYLEDONS. III



THE ORGANIZATION OF THE PRIMARY WALL IN DIFFERENTIATING CONIFER TRACHEIDS

By A. B. WARDROP*

[Manuscript received June 24, 1958]

Summary

In a study of differentiating tracheids of *Pinus radiata* evidence has been obtained which suggests that, in those cells with localized apical growth, surface enlargement takes place by the multi-net mechanism of wall growth. Features described, such as the difference in microfibril orientation on the inner and outer surfaces of the cell wall and the existence of well-developed corner thickenings, closely resemble similar features in elongating coleoptile parenchyma. It is argued that growth is not limited to the extreme tips of the cells as in root hairs, but that the growth zone extends some distance back from the cell ends.

I. INTRODUCTION

In the differentiation of conifer tracheids from the cambium, great interest attaches to the nature of the structural changes in the cell wall involved in the increase which takes place both in length and in breadth of the cells. For the greater part of this phase of surface enlargement of the differentiating tracheid only the primary wall surrounds the expanding protoplast, although certainly before enlargement is complete formation of the outer layer of the secondary wall begins (Wardrop and Dadswell 1952). Furthermore, the presence of numerous bifurcated and otherwise distorted tips of tracheids observed in macerated material indicates that tip growth does occur (Bannan and Whalley 1950; Wardrop and Dadswell 1952).

In most cases where the structural changes associated with surface enlargement have been studied, such as in seed hairs (Roelofsen and Houwink 1953) and in various types of parenchyma (Houwink and Roelofsen 1954; Wardrop 1955, 1956) the mechanism of multi-net growth proposed by Roelofsen and Houwink (1953) appears to operate. According to this concept microfibrils are deposited initially transverse to the major morphological cell axis and become progressively disoriented as growth proceeds; meanwhile new transversely oriented microfibrils are deposited on the inner side of the original expanding network. Thus, the final microfibril orientation on the outer surface of the cell wall reflects the extent and polarity of the growth which has occurred (Wardrop 1956).

The extension growth of conifer tracheids and angiosperm fibres differs from cases such as coleoptile and root parenchyma, because the tissues of which they are part do not extend in length and here localized tip growth is manifest. The question thus arises whether the multi-net mechanism applies in the cases where surface enlargement is far from uniform.

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It was established by van Iterson (1935) that the sign of birefringence of cambium is negative, indicating that the average micelle direction (microfibril direction) makes an angle with the cell length of more than 45 degrees. This conclusion was confirmed by X-ray diffraction studies (Preston and Wardrop 1949).

Initial electron microscopic investigations showed that in the cambium or its immediate derivatives the microfibrils have a characteristic felted appearance (Hodge and Wardrop 1950; Bosshard 1951, 1952; Preston and Ripley 1954; Svensson 1956), but in relatively few of the electron micrographs could any definite direction of microfibril orientation be recognized. In other investigations, however (Wardrop 1954), it was recognized that the microfibrils on the outer surface of the cells are arranged longitudinally near the cell tips but are considerably dispersed from this direction near the middle of the cells. Subsequently some evidence was obtained indicating a predominantly transverse microfibril orientation on the inner cell wall surface (Wardrop 1957). These observations led the author to speculate that the multi-net mechanism of Roelofsen and Houwink (1953) may also be operative during the dimensional changes involved in the differentiation of tracheids from the cambium since the observed microfibril orientation on the outer cell surface would be expected to reflect the extent and polarity of growth which had taken place in the primary wall during differentiation.

To ascertain if these conclusions are correct a more extensive examination of differentiating xylem was undertaken, and the results obtained, together with new observations relating to other aspects of primary wall structure, are described below.

II. MATERIALS AND METHODS

Differentiating xylem was collected from a tree of *Pinus radiata* following the method of Priestley, Scott, and Malins (1933). The tissue was then disintegrated in an homogenizer, or first treated to remove non-cellulosic wall constituents by alternate 30 minute extractions at 100°C in 1 per cent. sodium hydroxide and 1 per cent. hydrochloric acid before homogenization. The suspension of cell fragments was then mounted on collodion-covered grids before uranium shadow casting and examination in an R.C.A. type E.M.T. electron microscope.

III. RESULTS AND DISCUSSION

Two observations of the present series (Plate 1, Figs. 1, 2) support those made previously (Wardrop 1957) that the microfibrils on the inner surface of the cell wall are arranged transversely to the cell axis but are dispersed from this direction on the outer surface. That the orientation is transverse on the inner surface of a differentiating tracheid is clear from Plate 1, Figure 1, in which a developing pit border can be seen. It will be recalled from the observations of Sachs (1882) that the pit border begins to develop before general secondary thickening commences.

In Plate 1, Figure 2, part of a broken cell is shown so that both the outer (O) and inner (I) surfaces can be seen. From these additional observations, together with those already referred to, it is clear that in conifer tracheids, as well as in other cell types described above, there is a predominantly transverse arrangement of micro-

fibrils on the inner wall surface, and the microfibrils are dispersed to a variable degree from this direction on the outer surface. This observation parallels those which were made by Roelofsen and Houwink on other cell types and upon which the concept of multi-net growth, referred to above, was based. Furthermore, it is a consequence of such a mechanism that the degree of disorientation of the microfibrils from the transverse orientation is dependent upon the extent and direction of the growth which has taken place. Because the microfibrils on the outer surface are nearly parallel to the cell axis near the tips of the tracheids (Wardrop 1954), whereas a more random arrangement is observed near the middle of the cell (Plate 2, Fig. 1), it would appear justifiable to conclude that in differentiating xylem with localized tip growth the multi-net mechanism is operative.

The primary walls of differentiating xylem exhibit additional features which have been observed in other growing cells such as *Avena* coleoptile parenchyma (Wardrop and Cronshaw 1958), which must also be capable of explanation in terms of any concept of surface enlargement. In the oat coleoptile parenchyma, it was shown that there exist at the corners on the outer surface of the cells longitudinal bands of microfibrils, so that at these points the cell wall is thicker than elsewhere. It is of particular interest that in the primary walls of differentiating xylem these thickenings can also be seen (Plate 2, Figs. 1, 2). They do not have the appearance of bundles of strictly parallel microfibrils but appear to blend into the adjacent regions of the cell wall.

The pit fields in Plate 2, Figure 2, could be seen in many of the cell wall fragments examined (Plate 3, Fig. 1) together with regions penetrated by plasmodesmata (Plate 3, Fig. 2). In the view of Wardrop and Cronshaw (1958) these structures are important in that so long as the intercellular cytoplasmic strands pass through them cell wall formation in these regions is retarded. When the cytoplasmic strands are ruptured, however, then the points of penetration of the wall by plasmodesmata and the pit fields tend to become covered over by disorienting microfibrils from adjacent regions of the primary wall. In Plate 2, Figure 2, some evidence of this is seen in the pit fields between the two closer bands of longitudinal microfibrils.

The development of corner thickenings is attributed by Roelofsen (1958) to the operation of turgor forces between cells in the tissue, which would lead to a flow of material to the cell edges just as in air bubbles under pressure liquid is squeezed from the contact surfaces towards the edges. The observation that such corner thickenings are also present in meristematic cells prior to enlargement (Tupper-Carey and Priestley 1924) would also seem consistent with this point of view.

On the other hand, it was proposed by Wardrop and Cronshaw (1958) that because plasmodesmata and pit fields are concentrated on the surfaces of contact between cells the corners of the cells are regions where cellulose synthesis and microfibril orientation can proceed virtually unimpeded, so that these regions contain the oldest microfibrils of the wall.

Either or both of these viewpoints could apply to differentiating xylem as well as to coleoptile parenchyma. However, on the hypothesis that pit fields and plasmodesmata are absent from the cell corners, the occurrence of longitudinal corner thickenings can thus be interpreted in terms of the multi-net hypothesis. In addition

the extent and direction of growth itself during differentiation may be a significant factor. Thus, in Figure 1 (taken from Meeuse's (1941) paper) it can be seen that during differentiation the tangential walls become narrower and the radial walls wider, while near its middle the cell changes in cross section from rectangular to hexagonal form. When, for example, two adjacent walls expand radially then a

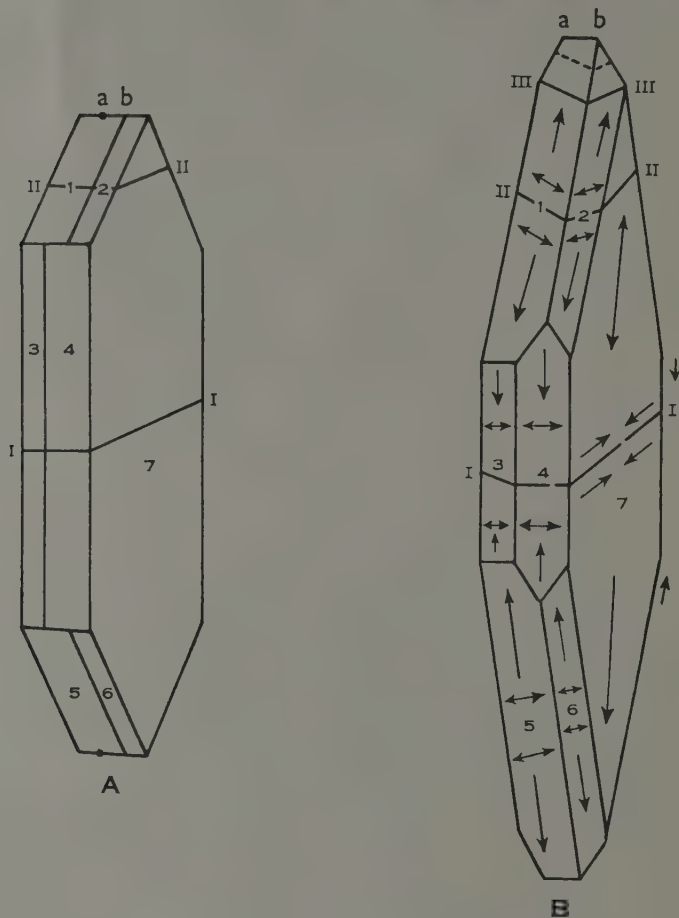


Fig. 1.—Diagrammatic representation of the change in form of a conifer tracheid during differentiation (from Meeuse 1941). *A*, a cambial initial; *B*, a mature tracheid. The cell length is reduced 50 times.

tendency for material to "pile up" at the corners can be appreciated, especially as the outermost microfibrils at this stage would, as Roelofsen (1958) points out, on the multi-net hypothesis, be carried passively by the enclosed protoplast.

The assessment of those factors which predominate in the development of the corner thickenings will depend on detailed studies both of cell wall organization and of cell form. However, at present, it can be seen that in differentiating tracheids, as well as in coleoptile parenchyma, the differences in microfibril orientation on the

inner and outer surfaces of the cell wall, the tendency for pit fields to be covered over during growth, and the presence of corner thickenings appear to be features not inconsistent with the multi-net mechanism.

Although the above observations appear to be consistent with the operation of multi-net growth during the differentiation of conifer tracheids, there remains the problem of how such a mechanism is involved in localized growth at the tips of the tracheids. Evidence of tip growth in differentiating tracheids has been reviewed by Bannan (1956). While the existence of tip growth (intrusive growth of Schoch-Bodmer (1945)) is not disputed the extent of localization of the terminal growth of the cell is not clear. In their discussion of multi-net growth Houwink and Roelofsen (1954) distinguish a number of cases in which there is a varying localization of deposition of cell wall material around the cell tip, with root hairs in which growth and deposition are localized at the extreme cell tip, and cotton hairs in which deposition apparently extends some distance away from the tip, as extreme cases. In the former case the resultant microfibril orientation on the outer cell surface was random, whereas in the latter there was a well-developed tendency towards axial orientation. In this respect the tips of differentiating fibres and tracheids resemble cotton hairs for here the microfibril orientation parallels the cell axis (Wardrop 1954, 1957). This does not imply, however, that as with cotton (O'Kelly 1953) the whole cell grows in length. Such a conclusion would imply that sliding growth as proposed by Krabbe takes place between the differentiating tracheids and, as pointed out by Frey-Wyssling (1950), such a concept is inconsistent with the continued cohesion of the tissue.

If, however, this resemblance to the growing cotton hair does indicate that the growth area of the cell is not confined to the extreme cell tip but extends some distance away from it then it may reflect a potentiality for growth both in length and in breadth in the regions beyond the "body" of the cell corresponding to its parent cambial initial (Amos, Bisset, and Dadswell 1950; Bosshard 1952). Such an extended region of growth could well account for the extraordinary form assumed by the ends of differentiating tracheids which may taper, bifurcate, alter the direction of their growth, or enlarge to balloon-like distensions (Wardrop and Dadswell 1952).

The extent of localization of growth to particular areas of the cell must, of course, reflect metabolic changes in these regions. There is some indication that these changes which cause increased plasticity of the wall (Heyn 1931) arise from a change in the metabolism of non-cellulosic cell wall constituents (Ordin, Cleland, and Bonner 1955). The properties of the wall are also influenced by the presence of ions such as those of calcium and potassium which have a hardening and softening effect respectively, and moreover, exchange between these ions in living tissue has been demonstrated by Cooil and Bonner (1957) and Tagawa and Bonner (1957).

These observations may give added significance to earlier observations of Dowding (1925) and of Macallum (1929) that growing cells tend to accumulate potassium ions in regions of growth. It is of interest to note that the observations of Sieber (see Busgen and Munch 1929) show that in spring, when differentiation proceeds most rapidly, calcium tends to be replaced by potassium in the cambium zone. In view of the work of Tagawa and Bonner (1957), presumably an exchange

could take place between potassium locally accumulated in the cytoplasm and calcium in the cell wall or intercellular layer. Such an exchange at the differentiating tracheid tip should lead to a locally increased plasticity of the wall, resulting in localized extension with consequent morphological changes in accordance with those which are described above and seem consistent with the multi-net mechanism. Although it is clear that much observation and experiment must be carried out to obtain data on the causes of localized growth in plant cells, the above points are perhaps worthy of consideration as a possible working hypothesis at the present time.

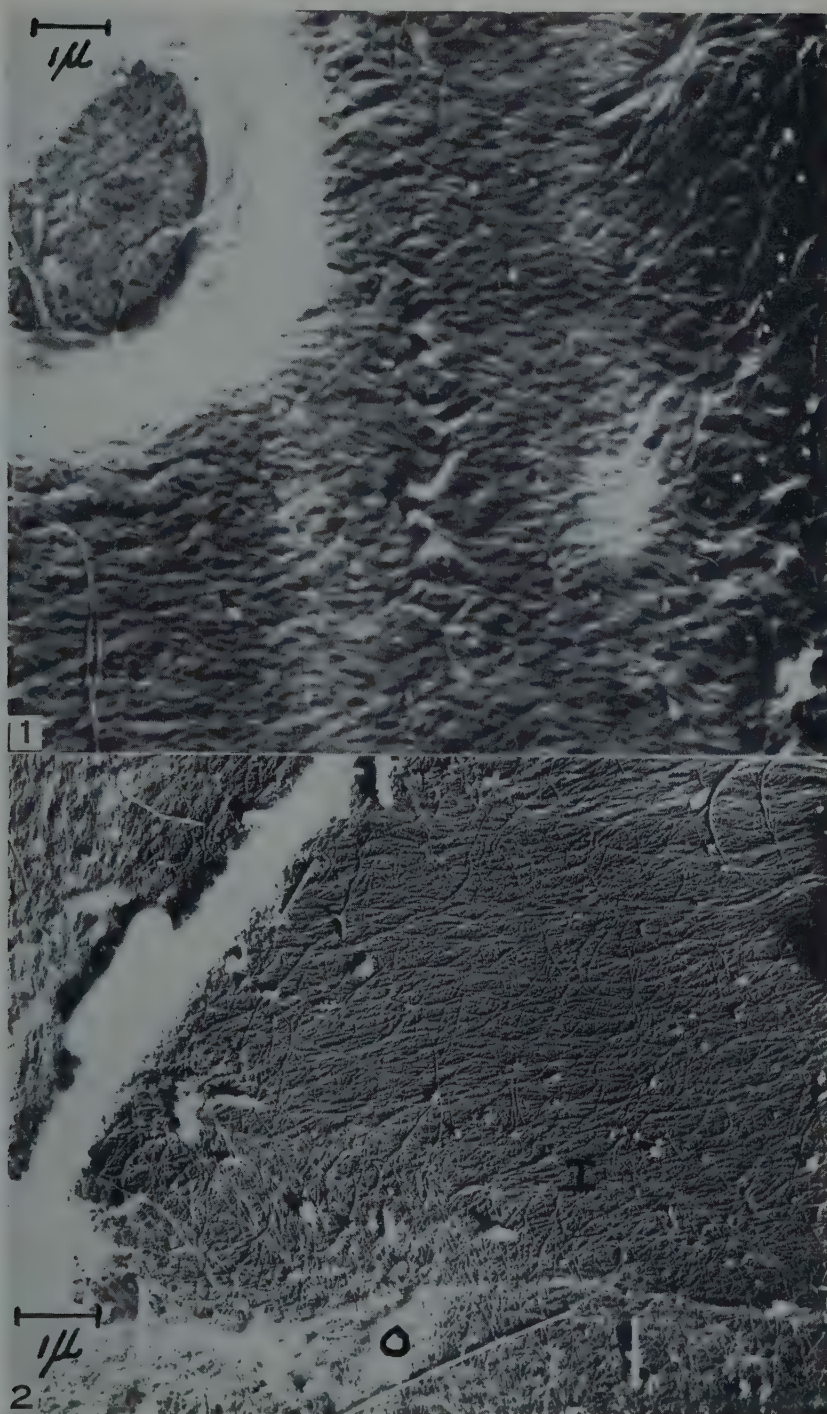
IV. ACKNOWLEDGMENTS

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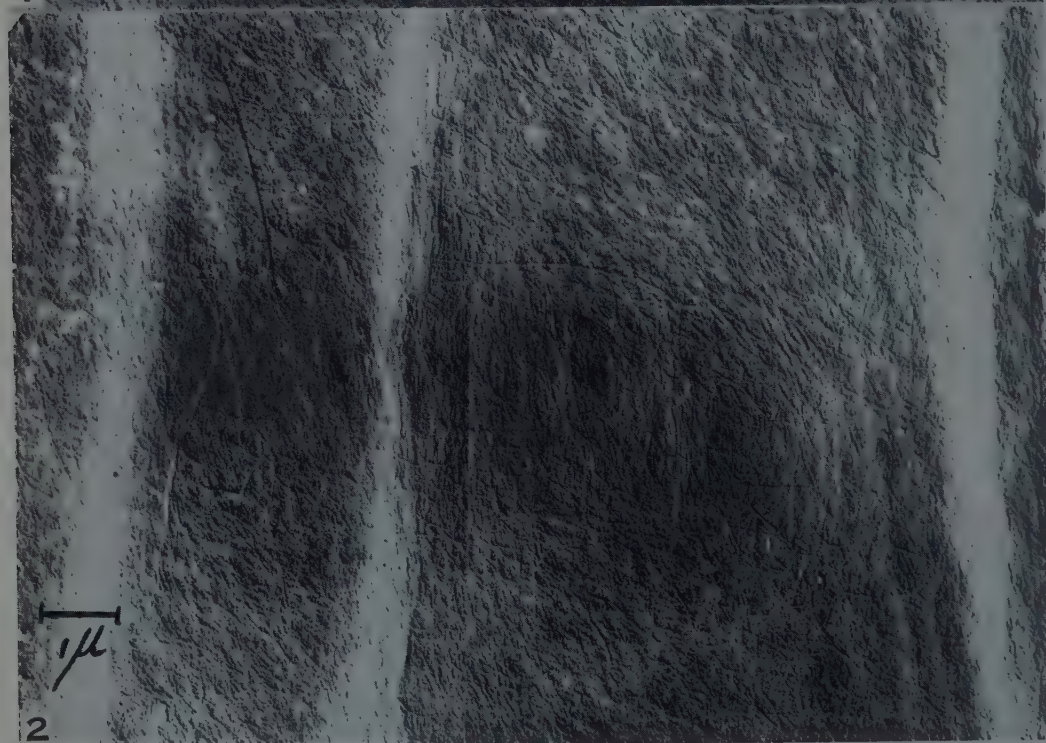
V. REFERENCES

- AMOS, G. L., BISSET, I. J. W., and DADSWELL, H. E. (1950).—*Aust. J. Sci. Res.* B 3: 393.
 BANNAN, M. W. (1956).—*Canad. J. Bot.* 34: 175.
 BANNAN, M. W., and WHALLEY, B. E. (1950).—*Canad. J. Res.* C 28: 341.
 BOSSHARD, H. H. (1951).—*Schweiz. Z. Forstw.* 12: 1.
 BOSSHARD, H. H. (1952).—*Ber. schweiz. bot. Ges.* 62: 482.
 BUSGEN, M., and MUNCH, E. (1929).—"Structure and Life of Forest Trees." (Chapman and Hall: London.)
 COOIL, B. J., and BONNER, J. (1957).—*Planta* 48: 696.
 DOWDING, E. S. (1925).—*Ann. Bot., Lond.* 39: 459.
 FREY-WYSSLING, A. (1950).—*Annu. Rev. Pl. Physiol.* 1950: 169.
 HEYN, A. N. J. (1931).—*Rec. Trav. Bot. Neerl.* 28: 113.
 HODGE, A. J., and WARDROP, A. B. (1950).—*Aust. J. Sci. Res.* B 3: 265.
 HOUWINK, A. L., and ROELOFSEN, P. A. (1954).—*Acta Bot. Neerl.* 3: 385.
 VAN ITERSON, G. (1935).—*Proc. 6th Int. Bot. Congr., Amsterdam, 1935.*
 MACALLUM, A. B. (1929).—*Proc. Roy. Soc.* B 54: 440.
 MEEUSE, A. D. J. (1941).—*Rec. Trav. Bot. Neerl.* 2: 387.
 O'KELLY, J. C. (1953).—*Plant Physiol.* 28: 281.
 ORDIN, L., CLELAND, R., and BONNER, J. (1955).—*Proc. Nat. Acad. Sci., Wash.* 41: 1023.
 PRESTON, R. D., and RIPLEY, G. W. (1954).—*J. Exp. Bot.* 5: 410.
 PRESTON, R. D., and WARDROP, A. B. (1949).—*Biochim. Biophys. Acta* 3: 549.
 PRIESTLEY, J. H., SCOTT, L. I., and MALINS, M. (1933).—*Proc. Leeds Phil. Lit. Soc. Sci. Sect.* 2: 365.
 ROELOFSEN, P. A. (1958).—*Acta Bot. Neerl.* 7: 77.
 ROELOFSEN, P. A., and HOUWINK, A. L. (1953).—*Acta Bot. Neerl.* 2: 218.
 SACHS, J. (1882).—"Textbook of Botany." (Clarendon Press: Oxford.)
 SCHOCH-BODMER, H. (1945).—*Ber. schweiz. bot. Ges.* 55: 313.
 SVENSSON, A. A. (1956).—*Ark. Kemi* 10: 239.
 TUPPER-CAREY, R. M., and PRIESTLEY, J. H. (1924).—*New Phytol.* 24: 156.
 TAGAWA, T., and BONNER, J. (1957).—*Plant Physiol.* 32: 207.
 WARDROP, A. B. (1954).—*Aust. J. Bot.* 2: 165.
 WARDROP, A. B. (1955).—*Aust. J. Bot.* 3: 137.
 WARDROP, A. B. (1956).—*Aust. J. Bot.* 4: 193.
 WARDROP, A. B. (1957).—*TAPPI* 40: 225.
 WARDROP, A. B., and CRONSHAW, J. (1958).—*Aust. J. Bot.* 6: 89.
 WARDROP, A. B., and DADSWELL, H. E. (1952).—*Aust. J. Sci. Res.* B 5: 385.
 WARDROP, A. B., and DADSWELL, H. E. (1953).—*Holzforschung* 7: 33.

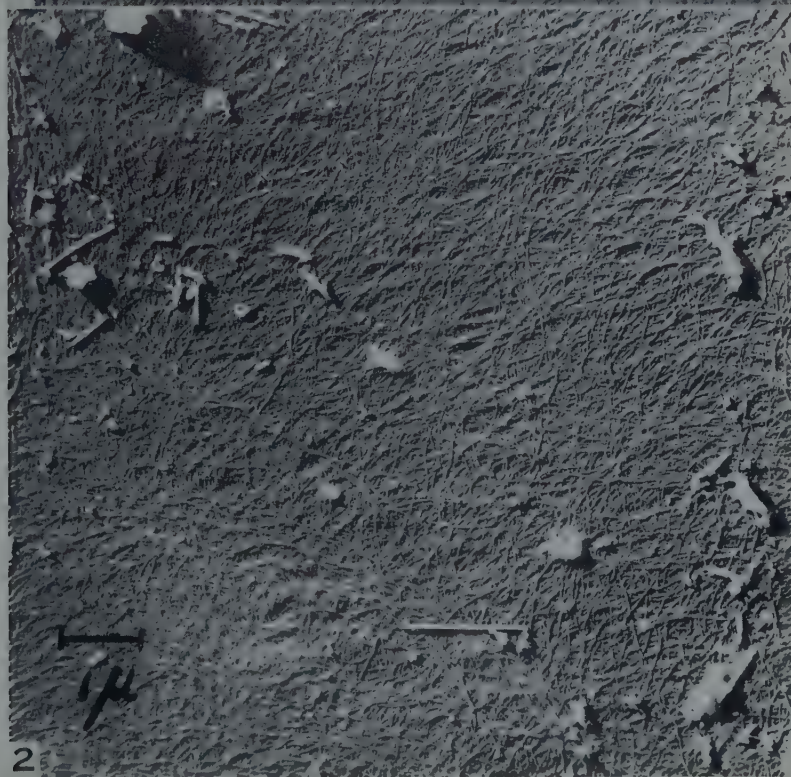
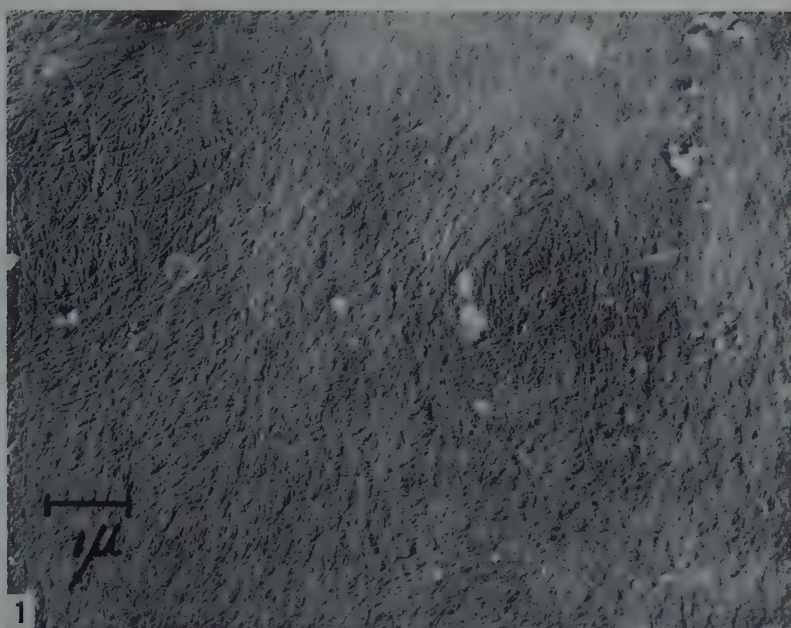
PRIMARY WALL GROWTH IN CONIFER TRACHEIDS



PRIMARY WALL GROWTH IN CONIFER TRACHEIDS



PRIMARY WALL GROWTH IN CONIFER TRACHEIDS



EXPLANATION OF PLATES 1-3

PLATE 1

- Fig. 1.—*Pinus radiata*. Inner surface of a differentiating tracheid showing predominantly transverse orientation of microfibrils and the commencement of formation of a bordered pit. Cell axis vertical. $\times 10,000$.
- Fig. 2.—*Pinus radiata*. Part of a differentiating tracheid broken open during maceration and showing part of the outer surface (O) and the inner surface (I). Cell axis vertical. $\times 10,000$.

PLATE 2

- Fig. 1.—*Pinus radiata*. The outer surface of differentiating tracheid showing two longitudinal (L) bands of microfibrils at the cell corners. Cell axis vertical. $\times 10,000$.
- Fig. 2.—As Figure 1, showing three bands of microfibrils with pit fields between them. Cell axis vertical. $\times 10,000$.

PLATE 3

- Fig. 1.—*Pinus radiata*. The presumed outer surface of a tracheid showing two pit fields. Cell axis vertical. $\times 10,000$.
- Fig. 2.—As Figure 1, showing points of penetration of the cell wall by plasmodesmata. Cell axis vertical. $\times 10,000$.

EFFECT OF SODIUM CHLORIDE ON LEAF SUCCULENCE AND AREA OF *ATRIPLEX HASTATA* L.

By R. F. BLACK*

[Manuscript received May 12, 1958]

Summary

The effect of NaCl in water cultures on morphology and histology are studied with *A. hastata*, a semisucculent halophyte with a mesomorphic leaf structure.

NaCl is shown to extend the ontogeny of the living leaf, and to produce an accelerated rate of leaf thickening, which has its main emphasis in the extended ontogenetic period. The most rapid thickening rate occurred when a high salt concentration (0.6M) was applied to only part of the root system, so as not to impede a rapid general growth rate in the plant.

Maximum leaf areas occurred in 0.1M NaCl cultures, minimum areas in 0.6M. Epidermal cell areas in a 0.2M treatment were double those of the treatment devoid of NaCl and those in the 0.6M treatment. Numbers of epidermal cells per leaf decreased progressively with increasing concentrations of NaCl.

The salt-induced thickening rate is looked upon as a process superimposed on a rather similar light- and moisture-sensitive process. Differences in timing between salt effects on leaf area and succulence are explained by differential vacuolation of epidermis and palisade tissue. The high-salt cultures (0.4–0.6M), which greatly reduced growth, apparently did not reduce turgor pressures necessary for succulence. It is considered that the data required to explain differences noted in epidermal cell size are relationships between their rates of expansion and the rates of maturation of structural limiting factors.

I. INTRODUCTION

Within the genus *Atriplex* two distinct types of leaf structure occur. One is mesomorphic with no water tissue within the mesophyll, and results in dorsiventral leaves of the normal dicotyledonous type. As far as is known, all the species of this type are facultative halophytes usually found in moist coastal salt-marshes, strand communities, and waste lands; *A. hastata* is certainly such a plant. The other type is a markedly xeromorphic structure with a water tissue occurring both as a dorsal and ventral hypodermis, and with a "Kranz" structure to the assimilating tissue. This is the structure found in our indigenous xerophytic species (Black 1954), and it is by far the most common in the genus (Moser 1934).

Species of both types exhibit succulence when NaCl is added to the substratum of the plant, but the more regular occurrence of succulence in the older leaves of *A. hastata* (Black 1956b, p. 77) makes it possible to apply a quantitative study of leaf measurements to this species.

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II. PLANT CULTURE

The leaf measurements and leaf material were obtained from plants grown in glass-house water cultures for another project on the ion uptake and growth of the species. The culture solutions were made up with distilled water and A.R. grade nutrient salts to the formula of solution 1 of Hoagland and Arnon (1938), and NaCl was then added in the required amounts. The solutions were contained in 4 litre enamelled cans and the plants were held in drilled pressed-wood covers.

Seedlings at the cotyledon stage were set out initially into cans containing from 0 to 0.1M NaCl. Those required for higher concentrations were moved up a concentration series with 2- to 4-day intervals between transfers. The 0.6M cultures were found to approximate to the maximum concentration of NaCl in which seedlings of *A. hastata* can be established, as a number of plants died when transferred to both the 0.5 and 0.6M solutions. The seedlings were subsequently thinned out to one per can and the solutions were occasionally aerated with a hand bubbler.

The first experiment consisted of a duplicate series of treatments of the following NaCl concentrations: 0.005, 0.01, 0.02, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6M. Plants were harvested in October after 16 weeks of growth in the water cultures. There was a marked inhibition of growth for NaCl concentrations above 0.3M (Black 1956*a*). The measurements and leaf samples necessary for the present work were collected during the last 2 weeks of the growth period.

In the second experiment a different seed batch was used. This was collected, as before, from near Woronora, N.S.W., but from a slightly different locality. The treatments used were duplicate 0 NaCl and 0.6M NaCl, quadruplicate 0.1M NaCl cultures, and two treatments in which the root systems were separated into two portions, one part being in a 0.1M and the other in a 0.6M culture solution. These plants were harvested in December, 2 days after leaf measurements and leaf samples had been taken. They had been growing in the water cultures for 11 weeks and had reached approximately the same morphological stage as the plants in the first experiment. Only the 0.6M plants suffered a significant inhibition of growth (Plate 1, Figs. 1, 2).

III. LEAF THICKNESS AND SUCCULENCE

(a) *Leaf Thickness Measurements*

(i) *Methods*.—Leaf thickness was measured with a dial micrometer calibrated in divisions of 0.001 inch. With suitably precise objects the instrument can be used to measure to the nearest 0.0001 inch. It can be used for thickness measurements on any turgid leaf, provided there are no prominent lateral veins to interfere with the placing of the measuring surfaces. With most leaves, reliance cannot be placed on a single reading.

A. hastata has opposite leaves in at least the lower vegetative nodes of each stem. Each node was taken as a unit structure on which leaf thickness measurements were based. Each leaf was measured once on either side of the midrib, approximately half way along the leaf. Thus the measurement for each node is a mean of four readings, of the thickness of two opposite leaves. These nodal readings

were expressed to the nearest 0.0005 inch. Mean lengths and breadths of these leaves were also determined for each node.

Measurements along any axis began from the youngest pair of leaves that could be conveniently measured with the micrometer (hereafter referred to as the youngest leaves) to the oldest pair not senescent. After senescence accurate measuring ceased to be possible because of the lack of turgor.

(ii) *First Experiment.*—Preliminary measurements were taken along lateral shoots of three plants. These figures (Table 1) showed that, with or without application of NaCl, thickening of the leaves proceeded with aging right up to the onset of senescence.

TABLE 1
LEAF THICKNESS MEASUREMENTS FROM LATERAL SHOOTS OF A.
HASTATA PLANTS

Each measurement (units 0.001 in.) is a mean of four readings on two opposite leaves; values below braces denote approx. range of constant leaf area

| NaCl Concn. of Culture Solution | Youngest Leaves —————> Oldest Leaves Measurable Not Senescent | | | | | | |
|---------------------------------------|--|----|------|------|------|-------|----|
| 0.0M | 11.5 | 11 | 11.5 | 12.5 | 13.5 | 14 | 15 |
| 0.1M | 14 | 14 | 17.5 | 18.5 | 18.5 | 20.5 | |
| 0.3M | 17 | 15 | 19 | 23 | 29 | 30.5* | |

*Smaller than other fully grown leaves.

In order to obtain a more comprehensive picture of the development of leaf thickness in the complete NaCl concentration series, measurements were taken along the central axis of each plant. The nodes were numbered from the base upwards, excluding the cotyledon scar. A mean was determined for the corresponding nodal measurements of the plants in duplicate treatments. The complete results are shown in Figure 1.

The lower heavy line indicates the thickness of the youngest leaves, together with their nodal numbers, for all treatments. The upper heavy line likewise gives the thickness and nodal numbers of the oldest leaves. The isonodal dotted lines illustrate leaf thicknesses at corresponding nodes throughout the treatments. The dropping of the isonodal lines between the 0.3 and the 0.4M NaCl treatments can be explained by the marked inhibition of growth that occurred in all treatments above 0.3M (Black 1956a).

The weakness of these data (Fig. 1) is the lack of a measure of the relative ages of the leaves of the various treatments, except for the salt levels from 0 to 0.1M where growth was very similar. Because of the greatly inhibited growth rate

of the high-salt plants ($0.4 \rightarrow 0.6M$ NaCl cultures), it could reasonably be expected that the youngest leaves of these plants were appreciably older than the youngest leaves of the low-salt plants. This could introduce a bias error sufficient to explain the increased thickness of these leaves in the high-salt plants (Fig. 1). Also, in spite of the fewer leaf nodes present in the high-salt plants, a more prolonged ontogenetic cycle of the leaves in these plants could explain the greater thickness of the oldest leaves, and the greater range of leaf thickness from these to the youngest leaves, in terms of leaf age alone. Thus for these plants, a salt-induced accelerated rate of thickening is not necessarily suggested by the data.

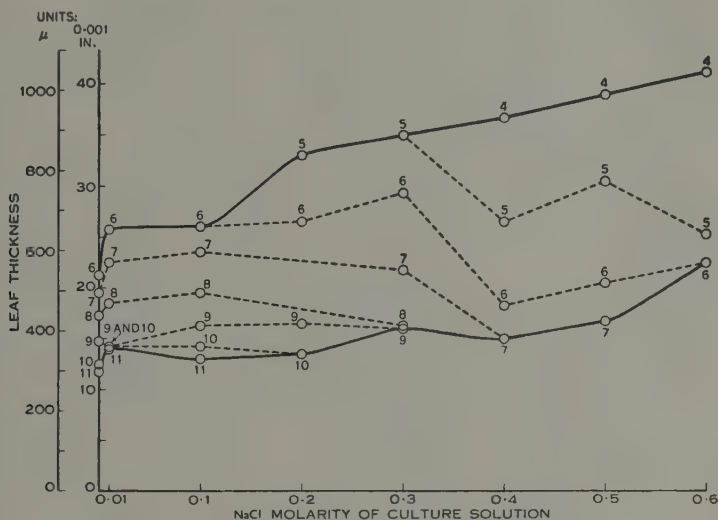


Fig. 1.—The total range of leaf thickness for the nodes at the central axes of *A. hastata* plants, from the oldest pair of leaves not senescent (upper heavy line) to the youngest pair of leaves measurable (lower heavy line), plotted against external NaCl concentration. Numbered points are means for the two corresponding nodal measurements of duplicate treatments. Nodes are numbered from the base upwards, excluding cotyledon scar.

(iii) *Second Experiment*.—Nodal means were determined for the central axes of the plants of the second experiment as for the first. The nodal means of the individual plants were plotted on the basis of the nodal numbers (Fig. 2) and tabulated (Table 2). These results show two important departures from those for the plants of the first experiment (Table 1, Fig. 1): first, the thicknesses of the youngest leaves were about the same for all treatments; and second, in the plants of the $0.1M$ NaCl treatments the leaves remained on more nodes than for the 0 NaCl treatments.

It is probable that the more rapid growth rate of the plants in the second experiment would largely account for these departures. Also trace NaCl in the 0 NaCl cultures was at a lower level in the second experiment, which might explain the comparatively earlier leaf senescence found in this treatment. In this experiment

the A.R. nutrient salts were purified for these traces. Small genotypic differences inherent in the different seed batches may also have played a part.

The treatments with both 0.1M and 0.6M NaCl cultures for the same plant are of particular interest. Owing to the dominant tap-root of this species, the only

TABLE 2
EFFECT OF NaCl TREATMENT AND POSITION AT THE CENTRAL AXIS ON LEAF THICKNESS AND LEAF AREA OF *A. HASTATA* PLANTS
Leaf thickness (T) in units of 0.001 inch and leaf area (A) of one face in sq. centimetres, both being means for the two opposite leaves of each node

| Treatment | Nodes Numbered from Base Upwards Excluding Cotyledon Scar | | | | | | | | | | | |
|------------------|---|---|-----|------|------|------|------|------|-------|-------|------|------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | |
| 0 NaCl A | T | — | — | — | — | — | 17 | 16 | 15.5 | 13 | 13 | 12 |
| | A | — | — | — | — | — | 12.5 | 12.5 | 11.9 | 12.3 | 10.0 | 7.0 |
| 0 NaCl B | T | — | — | — | — | — | 17 | 16 | 15 | 12 | 12 | 10.5 |
| | A | — | — | — | — | — | 11.2 | 13.8 | 11.4* | 7.9* | 8.9* | 8.0* |
| 0.1M A | T | — | — | 32 | 30 | 27 | 22.5 | 18.5 | 15.5 | 13 | 12 | — |
| | A | — | — | 7.7 | 10.0 | 12.4 | 14.0 | 13.7 | 14.6 | 10.9* | 9.6 | — |
| 0.1M B | T | — | — | — | 28.5 | 24 | 18.5 | 16.5 | 14.5 | 12.5 | 10.5 | — |
| | A | — | — | — | 11.8 | 13.0 | 16.6 | 16.7 | 16.7 | 14.5 | 12.1 | — |
| 0.1M C | T | — | — | 31 | 27.5 | 24 | 21 | 18 | 14.5 | 14 | 12 | — |
| | A | — | — | 8.1 | 12.9 | 14.1 | 17.0 | 16.6 | 14.8 | 14.2 | 11.0 | — |
| 0.1M D | T | — | — | 32 | 28.5 | 23.5 | 21 | 19 | 16.5 | 14 | 12.5 | — |
| | A | — | — | 10.0 | 15.1 | 15.7 | 17.4 | 17.6 | 15.4 | 14.4 | 12.0 | — |
| 0.1M and 0.6M | T | — | — | 50 | 40.5 | 30 | 21 | 17 | 15.5 | 14 | 11 | — |
| | A | — | — | 10.7 | 11.5 | 11.1 | 11.5 | 12.8 | 12.8 | 11.5 | 7.8 | — |
| 0.6M A | T | — | 48 | 36 | 29 | 21 | 14.5 | 11.5 | — | — | — | — |
| | A | — | 1.5 | 2.4 | 2.8 | 3.1 | 2.7 | 0.9 | — | — | — | — |
| 0.6M B | T | — | 44 | 37 | 30.5 | 22.5 | 14.5 | 12 | — | — | — | — |
| | A | — | 1.3 | 1.8 | 2.4 | 3.4 | 3.1 | 1.1 | — | — | — | — |

*Leaf shape distorted (pathological?).

method of dividing the root systems of the young plants was to loop up the tap-root to the base of the culture cover with a piece of cotton. This left the root system in two portions, one being a loop of the upper half of the tap-root, the other being the lower half hanging free. In one treatment the loop was placed in the 0.6M culture, while in the other treatment it was placed in the 0.1M culture. In both treatments most

of the subsequent root growth occurred in the 0.1M culture and there was a marked increase in the thickness of the older leaves compared with the 0.1M NaCl treatments. This increase in thickness was most marked in the compound treatment where the upper loop of the tap-root had been placed in the 0.6M culture. No doubt the reason for this was the greater absorbing area of the roots in the 0.6M culture in this treatment compared with the other, where only a poorly developed terminal portion of the tap-root hung into the 0.6M solution. The leaf thickness data of this latter treatment are not recorded here.

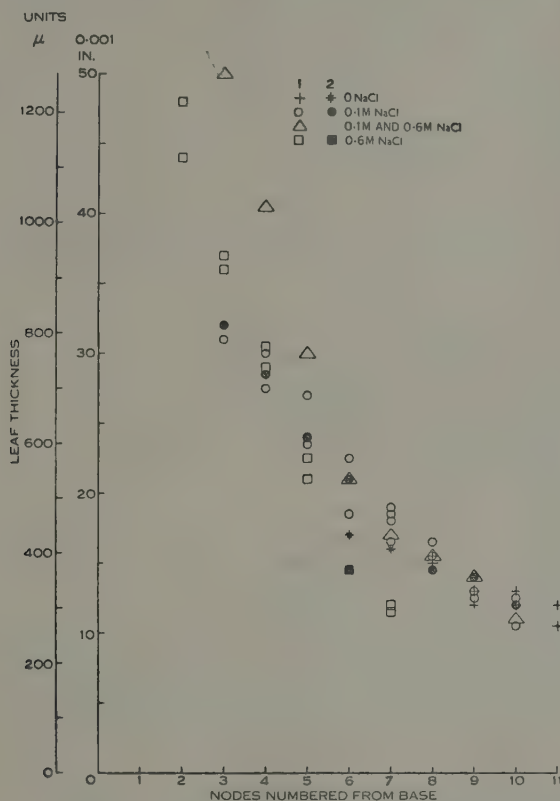


Fig. 2.—Leaf thickness for each node at the central axes bearing measurable leaves, for plants of *A. hastata* grown under four different NaCl treatments. Thickness is plotted against nodal numbers, which run from the plant base upwards, excluding cotyledon scar.

In all the 0.1M NaCl treatments of the second experiment, whether simple or combined with a 0.6M culture, the growth rates of the plants were very similar (Plate 1, Figs. 1, 2; Table 2). Thus the compound treatment (Fig. 2) shows that NaCl, applied to the roots of one of these plants, can induce an accelerated thickening rate of the leaves in a manner quite distinct from any secondary effects brought about by a greater life span of the leaves.

In order to obtain a measure of the actual thickening rates of the leaves of all treatments, the approximate age of each node in days was measured from the time

of its position at the first measurable node from the bud to the day when all measurements were taken. From the record it was calculated for the 0 NaCl plants that the last six measurable nodes formed at an approximately constant rate of one node every 7 days. In a similar manner, for all the 0.1M NaCl treatments, both simple and compound, the last eight measurable nodes formed at the approximate rate of one every 8 days. In the 0.6M NaCl treatments the oldest three measurable nodes formed at intervals of approximately 10 days, bringing the plants to the stage illustrated in Plate 1, Figure 1. Subsequently, the nodes formed at a slower rate of one every 14 days approximately. The increased rate in the early stages of these

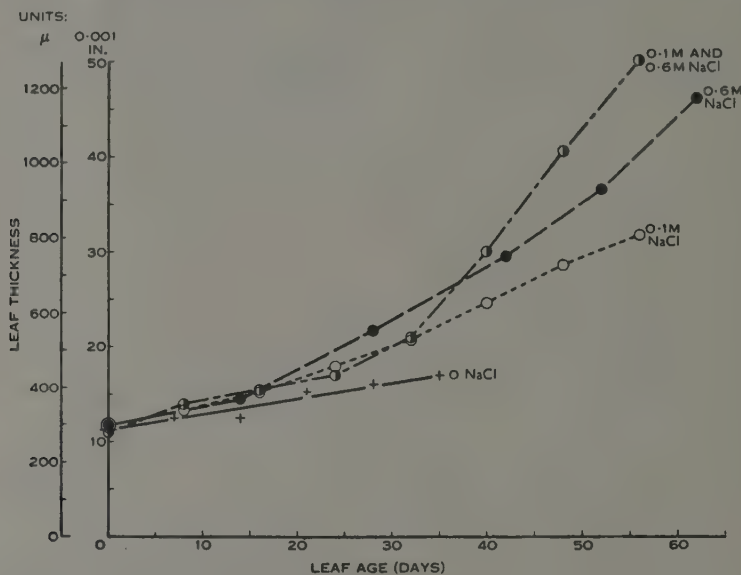


Fig. 3.—Mean leaf thickness for corresponding nodes at the central axes of replicate plants of *A. hastata*. Plants were grown under four different NaCl treatments. Thickness is plotted against the approximate leaf age from the time of their position on the first measurable node below the bud.

See Fig. 2 for complete replicate data.

plants was probably caused by their gradual transition from 0.1 to 0.6M cultures, through intermediate concentrations. The mean leaf thickness for each set of corresponding nodes of replicate treatments was determined, and these values were plotted on the basis of the calculated time intervals (Fig. 3).

The thickening of the leaves from the 0 NaCl treatment plants proceeded at an approximately constant rate of 0.00115 inch (29.2 μ) per week. Over the first fortnight of the ontogenetic cycle (commencing at the first measurable node) the thickening of the leaves of all treatments proceeded at about this same rate. From about this point on, an acceleration of the thickening rate occurred in all the NaCl treatments.

From the leaf area data (Table 2) it can be stated for all treatments that this first fortnight of development from the position at the first measurable node coincided approximately with the expansion of the leaves to their maximum area. Thus it

appears that a salt-induced acceleration of the thickening rate was delayed until the leaves had expanded to their full area.

The results (Fig. 3) for all the NaCl treatments show clearly that senescence was greatly delayed, and that during this extended ontogenetic period the salt-induced thickening rates reached maximum values. The average thickening rates for the different NaCl treatments, over this period from the 35th day to the onset of senescence, were as follows:

| | |
|---------------|-----------------------------------|
| 0.1M | 0.0032 in. (81 μ) per week, |
| 0.6M | 0.0053 in. (135 μ) per week, |
| 0.1M and 0.6M | 0.0085 in. (217 μ) per week. |

From the results of the first experiment (Fig. 1) it may be assumed that an accelerated thickening rate would be obtained by using water cultures of con-

TABLE 3
Na⁺, K⁺, AND Cl⁻ CONCENTRATIONS OF BULKED MATURE LEAF
SAMPLES FROM TREATMENTS OF SECOND EXPERIMENT

| Treatment | Concentration (m-equiv./l leaf water) | | |
|-------------------|--|----------------|-----------------|
| | Na ⁺ | K ⁺ | Cl ⁻ |
| 0 NaCl, A | 7.5 | 416 | 5.4 |
| 0 NaCl, B | 7.4 | 368 | 5.4 |
| 0.1M NaCl, A | 384 | 108 | 85 |
| 0.1M NaCl, B | 388 | 98 | 105 |
| 0.1M NaCl, C | 408 | 103 | 105 |
| 0.1M NaCl, D | 420 | 101 | 107 |
| 0.1 and 0.6M NaCl | 482 | 96 | 183 |
| 0.6M NaCl, A | 914 | 107 | 420 |
| 0.6M NaCl, B | 871 | 110 | 400 |

centrations as low as 0.01M NaCl. It is apparent that when using high NaCl concentrations, which by themselves would greatly reduce growth, the greatest acceleration is obtained by applying them to only part of the root system. In the example recorded here (Fig. 3) the final thickening rate was 7.4 times the basic 0 NaCl rate.

The internal Na⁺ and Cl⁻ concentrations for bulked samples of mature leaves from all treatments are given in Table 3. These show that the plant with the highest final thickening rate did not have the highest NaCl uptake, although it was appreciably above the 0.1M plants.

Summing up, NaCl when applied to the roots of *A. hastata* causes the production of thickened and succulent leaves in two ways: first, the salt causes the

leaves to have a greater life span, which by itself would increase their final thickness; and second, it induces an accelerated thickening process in the mature full-sized leaves. This latter process is most marked in the final ontogenetic stages of the living leaf, and when a high concentration of the salt is applied in such a manner as not to impede a rapid growth rate.

(b) Anatomical Changes

Differences between the old leaves of the high-salt treatments and the younger leaves of the same, or the old leaves of the 0 NaCl treatments, could readily be perceived by their thickness and "feel", the latter arising from their greater turgor and lack of flexibility. These leaves when bent snapped off sharply, in distinct contrast to the non-succulent leaves.

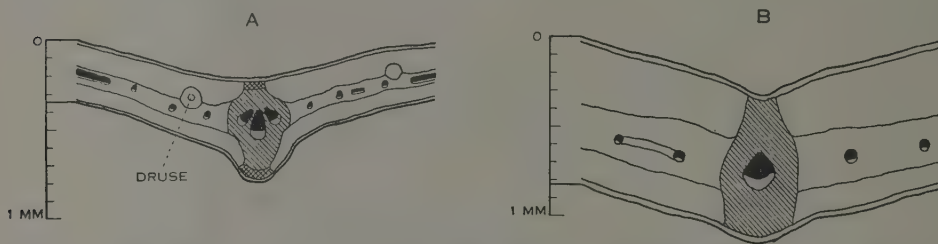


Fig. 4.—Transverse sections cut half way along midrib of a non-succulent and succulent leaf. Each was the oldest healthy leaf on the main axis: *A*, from 0 NaCl treatment; *B*, from 0.1 and 0.6M NaCl treatment. Parenchyma is singly cross-hatched; collenchyma, doubly. In vascular bundles, xylem is black and phloem white.

(i) *Vesicles*.—The salt-induced succulence of the whole leaf was in general paralleled by succulence in its constituent parts. This was brought out in a remarkable manner by the vesicular hairs present on the young leaves.

Young leaves from the first node below the terminal bud, and from many treatments of both experiments, were examined in surface view under the microscope. In all the 0 NaCl treatments, the vesicles were small and elongated into cylindrical, reniform, or dumb-bell shapes, with their long axes parallel to the epidermis. Their lengths averaged $90\ \mu$, and their widths $45\ \mu$. In the 0.005M NaCl treatments most of the vesicles were more rounded in shape and strikingly larger, being about $140\ \mu$ in length and $80\ \mu$ in width; there were some apparently mature vesicles much smaller in size and scattered among the larger ones.

The tendency to roundness was more consistently shown in the higher salt concentrations, where the vesicles were usually ovoidal or spherical in shape, and ranged from 100 to $200\ \mu$ at their longest axes.

(ii) *Leaf Structure*.—One reason for the brittle nature of the succulent leaves was found to be the progressive elimination of the mechanical tissue at the midribs and main lateral veins with the development of succulence. The young and old leaves of the 0 NaCl plants possessed ventral (adaxial) and dorsal (abaxial) collenchyma at the midrib (Fig. 4*A*), and the very young leaves of the high-salt plants were the same. As succulence developed, the ventral collenchyma was the first

eliminated, apparently by a stretching of the thickened walls as the cells increased in volume. In the very old succulent leaves the collenchyma was completely eliminated (Fig. 4*B*).

Succulence was caused by a volume increase in all the cells of the mesophyll (Fig. 5). There was no evidence of an increased number of cell layers forming the lamina. The proportion of the leaf volume occupied by air spaces decreased with

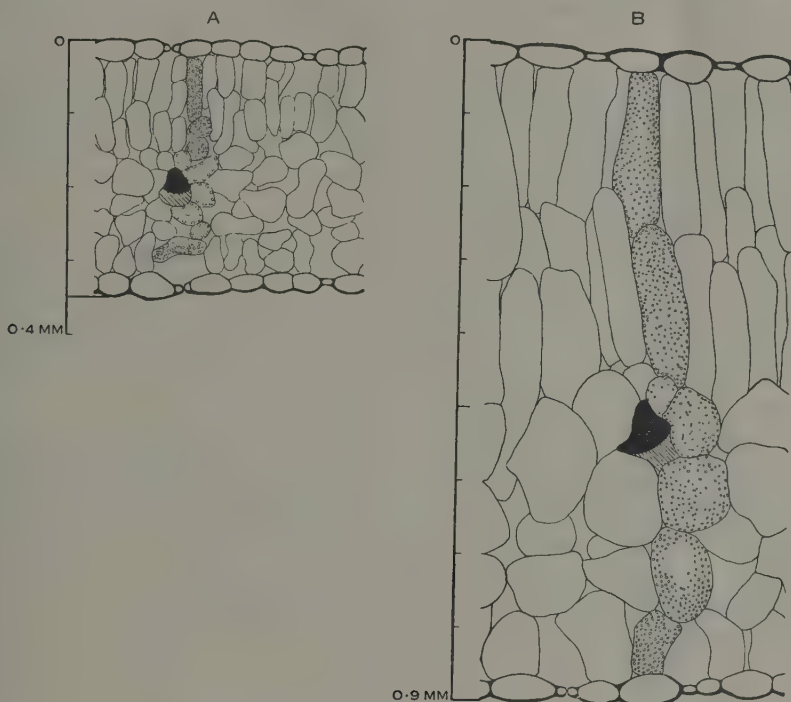


Fig. 5.—Cell detail in the lamina of a non-succulent and succulent leaf. Each was the oldest healthy leaf on the main axis: *A*, from 0 NaCl A treatment; *B*, from 0.1 and 0.6M NaCl treatment. Xylem is black, phloem singly cross-hatched.

succulence (Fig. 5). Sections of leaves from 0 NaCl treatments generally showed a number of druse-containing idioblasts at the base of the palisade mesophyll (Fig. 4*A*); these were not noticed in the very succulent leaves.

For the leaves examined, the chloroplasts were smaller and more numerous per cell in the very succulent leaves (Fig. 5). However, in the very young leaves of all treatments, the chloroplasts were similar in size and smaller than in the mature leaves. Thus the effect in the succulent leaves appeared to be a retarded development of the chloroplasts possibly through the multiplication of their numbers per cell.

IV. LEAF AREA AND EPIDERMAL CELL SIZE

The development of leaf area as a function of NaCl treatment and leaf age has already been mentioned for the plants of the second experiment (Table 2). It is clear

from these results that leaf area developed rapidly during an early stage in the ontogeny, when leaf thickness increased least.

In general, for plants treated with NaCl, the older mature leaves were smaller in area than the younger mature leaves (Table 2). The probable cause would be the younger "physiological age", with respect to leaf initiation, of these old leaves compared with the younger mature leaves of the same treatment (Ashby 1948a). Also it is possible that growth-inhibiting salt effects were greater in the younger plants.

For the plants of the second experiment, the maximum leaf areas occurred in the 0.1M NaCl treatments, while minimum areas resulted from the 0.6M cultures (Table 2). The 0.1M and 0.6M compound treatment caused a much smaller depression of leaf area development. The leaf area measurements for the plants of the first experiment showed similar salt effects.

Towards the end of the growing period of the first experiment, it was decided to investigate these salt-induced variations in leaf area with respect to the complementary factors of cell size and number. The epidermis was used because the product of the final surface areas and number of these cells is a measure of the final leaf area, and further, small pieces of this tissue could readily be stripped from mature fresh leaves for surface examination under the microscope.

The oldest healthy leaf on the main axis of each plant was removed, and its area (one face only) was determined. The results (Fig. 6A) for the leaves of the sixth node from the base of the 0 to 0.1M NaCl treatments, and from the fifth and fourth node of the higher salt treatments, decreased progressively in area with increasing concentration of NaCl. This decrease would have been largely caused by the direct growth-inhibiting effects of the salt. However, a small indirect effect of the salt may have been the possibly younger "physiological age" of the leaves from the lower nodes.

In a preliminary examination of pieces of epidermis stripped from various parts of both surfaces of an *A. hastata* leaf, it was noted that the epidermal cells were roughly the same size all over the leaf, and that stomates occurred with approximately equal frequencies on both surfaces. However, over the midrib and the main lateral veins, the epidermal cells were elongated in the direction of the veins. Pieces of epidermis were peeled from the central region of the leaves sampled, and cell counts were made using a squared ocular. Parts counted were selected only with regard to avoiding the elongated cells over the main veins. Separate counts were made of the stomates and the ordinary epidermal cells. Five counts were made on each of the ventral and dorsal surfaces, thus making 10 counts for each leaf.

With the single exception of the 0.4M NaCl treatment, there were no significant differences between the ordinary epidermal cell counts from the dorsal and ventral surfaces of the sampled leaves. Values for the stomatal index (Salisbury 1928) also did not vary significantly between either the dorsal and ventral surfaces of any one leaf, or the various salt treatments. The stomatal indices calculated lay between 22 and 28 per cent.

From the totals of the cell counts for each leaf, and including each stomate as a single cell, the mean area of the epidermal cells for each treatment was estimated.

These results (Fig. 6*B*) showed clearly that the cell area was approximately the same in the two extreme treatments, and that much larger cells resulted from some of the intermediate treatments. Thus the differences in leaf area (Fig. 6*A*) can by no means be accounted for by differences in epidermal cell size.

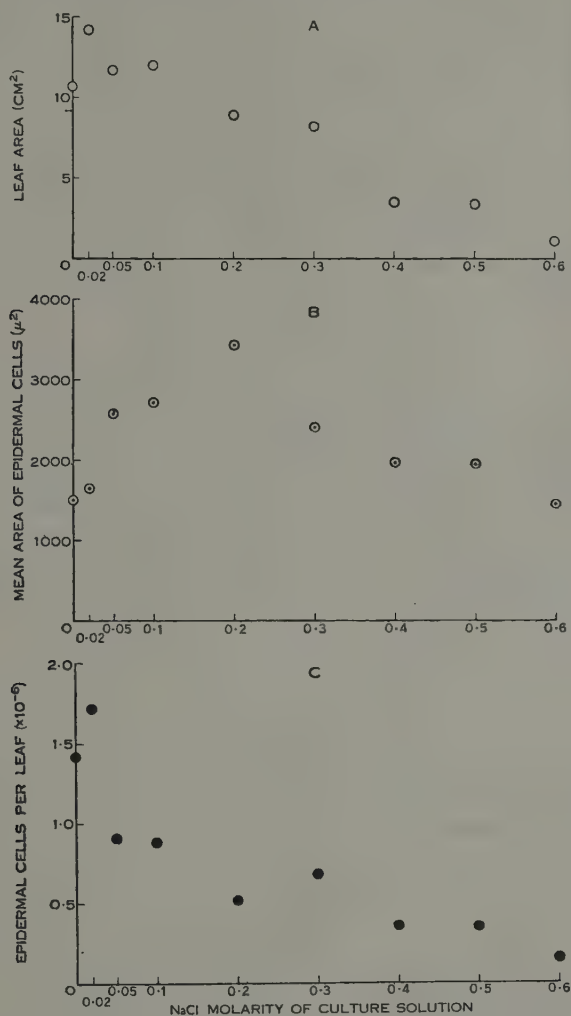


Fig. 6.—Data on leaf area, epidermal cell size, and cell number, of the oldest healthy leaf from the central axis of *A. hastata* plants, plotted against external NaCl concentration. *A*, leaf area (one face only); *B*, mean area of the epidermal cells including each stomate as a single cell; *C*, estimates of the total number of epidermal cells for both surfaces of each leaf, including each stomate as a single cell.

Finally an estimate was made for both surfaces of each leaf of the total number of epidermal cells, again including each stomate as a single cell. These estimates neglected epidermal cells at the edges of the leaves, and also any effects on the total

caused by the elongated epidermal cells situated over the main veins. This treatment of the results (Fig. 6C) clearly indicated a progressive inhibition of the meristematic activity of the dermatogen with increasing concentrations of NaCl in the culture solutions.

The small increases in leaf area from the 0 NaCl to the 0.1M NaCl treatments of the second experiment (Table 2) were apparently due to increases in cell size (Fig. 6B). These increases, however, were largely negated by a marked inhibition of meristem activity (Fig. 6C). The leaf from the 0.2M culture possessed the largest epidermal cells (Fig. 6B); yet the effect was more than offset by fewer cells being produced. In the three highest salt treatments, where both leaf areas and growth were greatly reduced (Fig. 6A, Black 1956a), epidermal cell size dropped back to that of the 0 NaCl treatment (Fig. 6B), and the reduced leaf area relative to the latter treatment was plainly caused by the very low number of cell divisions in each dermatogen (Fig. 6C).

V. DISCUSSION AND CONCLUSIONS

(a) "Physiological Age" Effects

In the *A. hastata* plants, the only noticeable differences between the vegetative leaves situated at different nodes on the main axes were their final full-sized areas. They increased in area from the base up to the last vegetative leaf free from an axillary inflorescence shoot. Over this range their shape at maturity did not vary, and their thickness at comparable ages appeared to remain constant as far as could be ascertained from observations made during the course of the experiments. This was the range of "physiological age" (Ashby 1948a) from which all measurements were taken.

The thickening rates calculated from Figure 3 depend on the assumption that leaves of different "physiological age" behave similarly. On theoretical grounds, a better method would have been to measure the thickness of only one leaf per plant at intervals of (say) 1 week. However, in practice it has been found that there is the danger of a slight residual crushing effect on the leaf after repeated measurements. Thus considering all the errors involved, the method used, of measuring all the leaves at the one time, was probably best.

(b) 0.1M-0.6M Sodium Chloride Compound Treatment

The highest thickening rate was obtained with this treatment. This probably explains why, on large and healthy plants in the field, extremely thick and succulent leaves are frequently found near the base of stems. It is reasonable to expect that in these habitats the NaCl concentration in the soil would vary widely from place to place within the root zone.

During the early stages of these compound treatments, when the size of the root system in each of the pair of cans was comparable, it was noted that most of the water uptake came from the 0.1M solution and that subsequently most of the root growth occurred in this same can, which in turn further increased the proportion of water withdrawn from the 0.1M can as the plant developed. These observations agree with the work of Eaton (1941). Thus the 0.1M solution was the main source of water for the plant, and the 0.6M solution acted as an extra source of NaCl (Table 3).

(c) *Light and Moisture Effects*

It has been shown that leaves increase in thickness by elongation of the palisade cells, under the influence of light, aridity, or both (Lothelier 1893; Eberhardt 1903; Hesselman 1904; Clements 1905; Hanson 1917; Watson 1942; Cormack 1955). In salt treatment, a similar increase in thickness occurs in leaves of *A. hastata* and wallflower plants (Boodle 1904).

It is suggested that salt-induced thickening can be looked upon as a process superimposed on a rather similar light- and moisture-sensitive one. There is, however, a difference of timing exhibited in the *A. hastata* plants, the salt-induced process having its main emphasis late in an extended ontogenetic period. It was this similarity in the responses of palisade cells to NaCl and light that prompted Warming (1909, p. 220) to state that "... common salt acts morphologically approximately in the same manner as sunlight".

(d) *Timing of Cell Size Increases*

For leaves in general, the epidermal cells are the first to vacuolate, and the palisade cells are the last (e.g. Avery 1933; Black 1954). If it is assumed that a salt-induced enlargement of a cell is delayed until vacuolation takes place, then the differential vacuolation of the epidermis and palisade tissue would explain why salt succulence is produced in leaves of *A. hastata* after salt effects on leaf area have become fixed.

Some grounds for this assumption are given by the salt uptake characteristics of vacuolated and non-vacuolated cells, since the work of Steward and Millar (1954) showed that non-dividing cells with expanding vacuoles took up more ^{137}Cs than actively dividing cells.

(e) *Yields and Succulence*

The reduced growth in the plants of the high-salt treatments of both experiments (Plate 1; Black 1956a), was probably caused by the high osmotic pressures of these solutions (Magistad 1945). These osmotic tensions were certainly in evidence when the seedlings were transferred to the high-salt cultures, as the plants frequently wilted for a time. If an abrupt change was made from a low- to a high-salt treatment the seedlings died, apparently from desiccation.

The leaf thickness results (Figs. 1-3) clearly show that the osmotic tensions of the high-salt cultures did not prevent the turgor pressures necessary for the development of succulence in the mesophyll; also, greatly enlarged vesicles were present on the very young leaves of the high-salt plants.

(f) *Leaf Area and Cell Size*

The increased area of the epidermal cells with salt treatments up to 0.2M NaCl (Fig. 6B), agree with what has been observed of the effects of NaCl on the mesophyll cells. The decreased epidermal cell areas for higher salt treatments suggest that some mechanism was limiting cell size development in this tissue, as cell enlargement continued in the mesophyll of the old leaves and the vesicles of the young leaves.

The limiting factors were probably structural changes such as cell wall and cuticle thickening and hardening. The maturation of the vascular system may also have played a part. Thus the evidence required to explain the differences noted in epidermal cell size is the relationships for the various treatments between the rates of expansion of the vacuolated epidermal cells and the rates of maturation of the structural limiting factors.

(g) Leaf Area and Cell Number

The reduced numbers of epidermal cells per leaf for the three highest salt treatments (Fig. 6C) were possibly effects of the high osmotic tensions or physiological dryness of these culture solutions. These responses could be equivalent to those noted on the reduced number of epidermal cells in the leaves of *Ipomoea* brought about by physically low moisture treatments (Ashby 1948b). On the other hand, the big drop in epidermal cell number from the 0 NaCl to the 0.2M culture solution may indicate a more specific chemical inhibition of the meristematic activity of the dermatogen.

VI. ACKNOWLEDGMENTS

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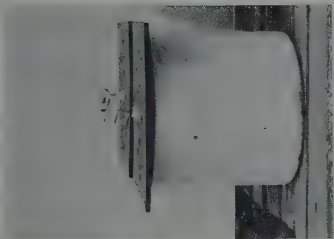
The subject matter of this paper represents a part of work submitted in a thesis for the Ph.D. degree of the University of Sydney.

VII. REFERENCES

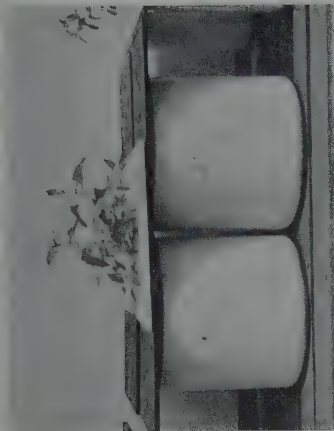
- ASHBY, E. (1948a).—Studies in the morphogenesis of leaves. I. An essay on leaf shape. *New Phytol.* **47**: 153–76.
- ASHBY, E. (1948b).—Studies in the morphogenesis of leaves. II. The area, cell size and cell number of leaves of *Ipomoea* in relation to their position on the shoot. *New Phytol.* **47**: 177–95.
- AVERY, G. S. (1933).—Structure and development of the tobacco leaf. *Amer. J. Bot.* **20**: 565–92.
- BLACK, R. F. (1954).—The leaf anatomy of Australian members of the genus *Atriplex*. I. *Atriplex vesicaria* Heward and *A. nummularia* Lindl. *Aust. J. Bot.* **2**: 269–86.
- BLACK, R. F. (1956a).—Effect of NaCl in water culture on the ion uptake and growth of *Atriplex hastata* L. *Aust. J. Biol. Sci.* **9**: 67–80.
- BLACK, R. F. (1956b).—Leaf xeromorphy, mineral nutrition, salt uptake and water relations in the genus *Atriplex*. p. 77. Ph.D. Thesis, University of Sydney.
- BOODLE, L. A. (1904).—Succulent leaves in the wallflower (*Cheiranthus cheiri* L.). *New Phytol.* **3**: 39–46.
- CLEMENTS, EDITH S. (1905).—The relation of leaf structure to physical factors. *Trans. Amer. Micr. Soc.* **26**: 19–102.
- CORMACK, R. G. H. (1955).—The effect of extreme shade upon leaf form and structure in *Vicia americana*. *Canad. J. Bot.* **33**: 293–7.
- EATON, F. M. (1941).—Water uptake and root growth as influenced by inequalities in the concentration of the substrate. *Plant Physiol.* **16**: 545–64.

LEAF SUCCULENCE AND AREA OF ATRIPLEX HASTATA

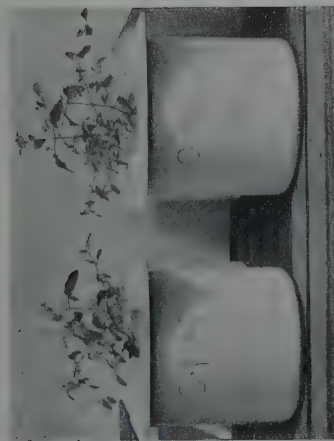
C



B



A



1



2

- EBERHARDT, PH. (1903).—Influence de l'air sec et de l'air humide sur la forme et sur la structure des végétaux. *Ann. Sci. Nat., Bot.* (8)18: 61–153.
- HANSON, H. C. (1917).—Leaf structure as related to environment. *Amer. J. Bot.* 4: 533–60.
- HESELMAN, H. (1904).—Zur Kenntnis des Pflanzenlebens schwedischer Laubwiesen. *Beih. bot. Zbl.* 17: 311–460.
- HOAGLAND, D. R., and ARNON, D. I. (1938).—The water culture method for growing plants without soil. *Circ. Calif. Agric. Exp. Sta.* No. 347: 1–39.
- LOTHELIER, M. A. (1893).—Recherches sur les plantes à piquants. *Rev. Gén. Bot.* 5: 480–3, 518–28.
- MAGISTAD, O. C. (1945).—Plant growth relations on saline and alkali soils. *Bot. Rev.* 11(4): 181–230.
- MOSER, H. (1934).—Untersuchungen über die Blattstruktur von *Atriplex*—Arten und ihre Beziehungen zur Systematik. *Beih. bot. Zbl.* 52: 378–88.
- SALISBURY, E. J. (1928).—On the causes and ecological significance of stomatal frequency, with special reference to the woodland flora. *Phil. Trans. B* 216: 1–65.
- STEWART, F. C., and MILLAR, F. K. (1954).—Salt accumulation in plants: a reconsideration of the role of growth and metabolism. *Symp. Soc. Exp. Biol.* 8: 367–406.
- WARMING, E. (1909).—“Oecology of Plants.” p. 220. (Clarendon Press: Oxford.)
- WATSON, R. W. (1942).—The mechanism of elongation in palisade cells. *New Phytol.* 41: 206–21.

EXPLANATION OF PLATE 1

Fig. 1.—*A. hastata* plants of second experiment after 1–5 weeks of growth in final NaCl treatments. *A*, right, 0 NaCl treatments; left, 0.1M *A* and *B* treatments. *B*, 0.1M and 0.6M compound treatments with divided root systems. *C*, 0.6M treatments.

Fig. 2.—The same plants 5 weeks later and 1 week before leaf measurements were taken.

THE STATISTICAL STUDY OF PLANT DISTRIBUTION PATTERNS USING A GRID OF QUADRATS

By H. R. THOMPSON*

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Summary

The statistical theory of the method of analysis of variance on a grid of contiguous quadrats is examined and the results from theoretical models for plant communities giving rise to non-randomness in the field discussed. Some field data are analysed and the theoretical and practical results correlated to determine the efficiency of the method as a practical technique.

The large element that chance variation plays in practice makes it essential for several samples of the same community to be taken so that real and chance effects can be distinguished. A knowledge of the morphology of the species under consideration is desirable, for then some idea of the expected analysis of variance is available and there should be no confusion between different models.

I. INTRODUCTION

Greig-Smith (1952) has pointed out the advantages to be gained from using contiguous quadrats in the study of the structure of plant communities instead of the older method of quadrats scattered at random. Ecologists wishing to obtain information on the way environment and other factors influence the spread and distribution of species and using this latter method often found themselves hindered by the non-random distributions encountered in the field, especially if only presence and absence of the given species was being recorded. With an actual count of the number of plants in each quadrat it is possible to test the observed distribution against some theoretical non-random distribution like the negative binomial distribution, say, but this type of approach is of limited value because it ignores the correlational and distributional pattern of neighbouring plants. We may say that while certain quantitative information is available from the use of random quadrats, its interpretation is strictly limited. In some cases, of course, the nature of the non-randomness may be obvious from a superficial inspection, but there will remain many cases in which the detection of an existing mosaic pattern due, say, to the presence of different phases in a community, or to competition, will present much greater difficulty. The random quadrat method will indicate only the degree, and perhaps the type, of non-randomness present, but will give no clues to the nature of more complex patterns.

It has generally been found in practice that the individual plants of a species tend to aggregate in patches or clumps, a property described as "overdispersion"

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from the fact that the ratio of variance to mean in a sample of quadrats is greater than unity, the expected value on the assumption of a Poisson distribution. Overdispersion may be caused (Clapham 1936) by the vegetative reproduction of a species in which offspring plants cluster round the parent (with seeds, the overdispersion should normally be less), or by a mosaic structure of the vegetation in which the units of the mosaic are patches of individual plants, the units being in a more or less irregular pattern. The corresponding property of underdispersion, in which the ratio is less than unity, has also been encountered, usually because of the effect of competition between individuals, tending to produce a more even distribution than the Poisson. It is sometimes the result of a regular soil fertility pattern, with suitable patches regularly spaced, or the exclusion of subordinate plants close to a dominant.

The type of dispersion varies with the size of quadrat used: quadrats equal in size to the patches of a mosaic would give strong overdispersion, while a quadrat size equal to that of a recurrent pattern would result in strong underdispersion. This fact is of great importance in the application of the method of sampling by a grid of contiguous quadrats. Adjacent quadrats or groups of quadrats may be combined into blocks, which are effectively larger-sized quadrats, in such a way that blocks of a given size all contain the same number of blocks of a smaller size, and the sums of squares appropriate to each block size may be calculated in an analysis of variance. The set of variances (mean squares) obtained will indicate whether any particular size of block forms a unit of some larger-scale heterogeneity than would be detectable from single quadrats scattered at random. While for a random distribution of plants the variance should be the same for all block sizes (equal to the mean number of plants per quadrat, in fact), for a block size equal to the unit of heterogeneity in an overdispersed community it is much greater than the mean, and for a block size equal to the size of a recurrent pattern (underdispersion) it is less.

Greig-Smith (1952) carried out an empirical investigation into the efficiency of the grid method, constructing artificial communities of plants by means of coloured discs scattered by hand over a grid of quadrats marked on the floor. In different experiments he attempted to reproduce two types of situation occurring in practice, one producing overdispersion (aggregation of individuals in randomly scattered clumps), and the other a mosaic of areas with the phases containing plants distributed at random with different densities. He plotted the variances calculated for each model against block size, and generally found peaked graphs, the occurrence of these peaks being correlated with such variables as area and density of clumps of plants. It will be more convenient to discuss his conclusions in detail later, in the light of the theoretical results obtained in this paper. It may be pointed out, however, that because of his empirical approach, Greig-Smith had no proper knowledge of the expected variances for his models, and the absence of repetitions for each model meant that no indication was available of the sampling variation. In view of the possible value of the method as a practical field technique, it seemed worth while to test the validity of his assertions by determining expected analyses

of variance for a number of theoretical models, at the same time investigating the accuracy, or reliability, of the method by a study of the sampling errors involved.

Various models for plant communities are derived in this paper subject to the requirements of two opposing factors, the suitability of the model as a representation of what would be likely to occur in the field, and the practicability of handling the model mathematically. We consider models producing overdispersion of individuals through the clustering of offspring round parent plants, in which the clusters or groups are themselves random or overdispersed, or alternatively underdispersed, these representing different stages in the development of a community in accordance with the concepts of Clapham (1936) and Ashby (1948). Another model results in underdispersion of individuals because the distances of neighbouring plants from each other obey a function in which the probability of too short distances occurring is small. Also, following Greig-Smith (1952), we consider theoretically models in which the plants are distributed at random but with different densities in a mosaic of areas over the grid. The mathematical theory for the derivation of expected values and sampling distributions of the individual terms of the analysis of variance on a grid was treated in detail by Thompson (1955) and will not be given again here. The only assumption made there which might affect the ecological application is the assumption that the individual plant has negligible area, and the degree of approximation involved will not be great if the size of the plant is fairly small in comparison with the quadrat size. This is certainly the case with some plants, in particular those studied by Phillips (1954), using Greig-Smith's methods.

The theoretical results are used to analyse in greater detail some available field data which have either used the grid method or are amenable to its application. Under the first category falls the (1954) investigation of Phillips on the ecology of *Eriophorum*, a characteristic species of bogs and wet moorlands, which was planned with the direct intention of applying the analysis of variance technique. Several sample grids were taken from communities or habitats in which *Eriophorum* was an important though not necessarily predominant constituent, and only the analyses of variance on these grids were published. An examination of these results is made in conjunction with a study of the morphology of the species, and the validity of Phillips's conclusions tested against the theory. The other available data, those of Cain and Evans (1952), are in the form of counts of plants of three species of herbs in contiguous quadrats over a large field, admirably suited to the application of the grid method. The fully mapped distributions of the individual plants are given by them, and for the purposes of this paper sample grids were selected from suitable parts of the field and the analyses of variance calculated. The theoretical conclusions drawn from these results are correlated with the distributional properties of the species as described by these authors.

II. THEORETICAL MODELS

(a) *Analysis of Variance on a Grid*

The size of the grid to be used in the field is quite arbitrary, apart from considerations of convenience and the amount of information available; in this paper

it is taken to contain 256 quadrats, arranged in a 16×16 square. This was considered large enough for practical and theoretical purposes, for if the quadrat size is suitably chosen, all the important ecological effects should appear in the terms for the smaller-sized blocks, which have a fairly large number of degrees of freedom and are consequently subject to less chance fluctuation. Greig-Smith (1952) used a grid of 512 quadrats in his empirical investigation while Phillips (1954) used either 256 or 512 quadrats in her application of the method. The choice of 256 quadrats is to a certain extent based on their experience.

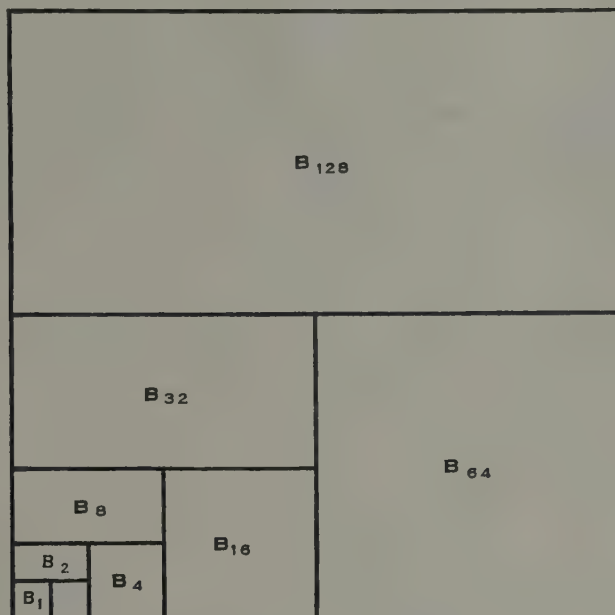


Fig. 1.—Arrangement of blocks on a grid.

The whole grid is divided into blocks of 2, 4, 8, 16, 32, 64, and 128 quadrats as indicated in Figure 1, blocks of the same size always having the same shape. It is to be noted that the rectangular blocks always have their longer side in the same direction; it is immaterial whether this direction is horizontal (as in Fig. 1) or vertical if no trends are present, but whichever way is chosen, it should be kept so consistently throughout an investigation. For the analysis of variance on this grid we form the block totals for all sizes of blocks. If $B_{k(i)}$ is the total number of plants in the i th block of k quadrats ($i = 1, 2, 3, \dots, 256/k$) then we calculate the series of sums of squares $S_1, S_2, S_4, S_8, \dots, S_{256}$ by means of the formula

$$S_k = \frac{1}{k} \sum_{i=1}^{256/k} B_{k(i)}^2.$$

We note that B_{256} is merely the total number of plants over the grid. The analysis

of variance, giving the variances attributable to each block size, is then quite simply obtained, the necessary calculations being shown in Table 1.

Plotting the observed set of variances V_k against k on a graph, as suggested by Greig-Smith (1952), we have a useful visual method of presenting outstanding features of the analysis such as peaks or trends, or determining approximately which type of model the community most closely resembles. With some theoretical models it is possible to calculate the limits of error of individual variances and determine "significance bands" for each set outside which variances observed from samples of the model would only be expected to fall with a given probability, say 0.05; for these cases we can test empirically the departures of an observed from a theoretical set of variances. Significance bands are discussed by Thompson (1955) and their meaning and use will become more clear as particular cases are treated.

Greig-Smith (1952) uses the graphical method in conjunction with the F test to find significantly high variances, testing each V_k ($k > 1$) against V_1 . The F test is

TABLE 1
ANALYSIS OF VARIANCE FOR A GRID OF 256 QUADRATS

| Block Size (k) | Degrees of Freedom (n_k) | Mean Square (V_k) | Block Size (k) | Degrees of Freedom (n_k) | Mean Square (V_k) |
|-----------------------|---------------------------------|--------------------------|-----------------------|---------------------------------|--------------------------|
| 1 | 128 | $(S_1 - S_2)/128$ | 16 | 8 | $(S_{16} - S_{32})/8$ |
| 2 | 64 | $(S_2 - S_4)/64$ | 32 | 4 | $(S_{32} - S_{64})/4$ |
| 4 | 32 | $(S_4 - S_8)/32$ | 64 | 2 | $(S_{64} - S_{128})/2$ |
| 8 | 16 | $(S_8 - S_{16})/16$ | 128 | 1 | $(S_{128} - S_{256})$ |

generally unsuitable, however, because the assumptions needed for its proper application will hardly ever be satisfied in practice. On the null hypothesis that the numbers in the quadrats are independently and normally distributed with the same variance, the ratio V_k/V_1 follows the F distribution with n_1 and n_k degrees of freedom, and its expected value is unity. This hypothesis will usually be violated because of the non-independence and non-normality produced by the associations of plants into clumps. With most of the models discussed in this paper the expected value of the F ratio is much greater than unity, and so the occurrence of a significant ratio in a sample analysis of variance is often meaningless. Significant results can be obtained where none actually exist.

(b) *Random Distribution of Plants*

It is convenient to discuss first the model in which the plants are distributed independently of each other, i.e. at random, chiefly because it is the simplest assumption that can be made about a community, and also because it forms the

basis of many of the more complicated models. It is well known that complete randomness is hardly ever encountered in the field, but it might be expected to occur when an area is first invaded by a species, especially if it is colonized by wind-borne seeds. It is also useful to be able to test how far a given observed distribution of plants departs from a purely random distribution.

With a random (Poisson) distribution of plants, the analysis of variance on a grid has one outstanding feature: all variances are expected to be equal to the mean number of plants per quadrat m , so that we have

$$E(V_k) = m$$

for all k , the left-hand side of the equation indicating the expectation or mean value of the variance V_k . If variance is plotted against block size we should ideally obtain a set of points on a horizontal straight line. Such a circumstance would hardly be likely to occur in practice, however, even if the plants did obey a Poisson distribution, and the significance band method is of value here in giving some idea of the extent of the chance variations possible in a sample analysis of variance.

TABLE 2
SIGNIFICANCE BANDS FOR V_k/m FOR A POISSON DISTRIBUTION

| n_k : | 256 | 128 | 64 | 32 | 16 | 8 | 4 | 2 | 1 |
|----------|------|------|------|------|------|------|------|------|------|
| 95% band | 0.81 | 0.75 | 0.67 | 0.56 | 0.42 | 0.27 | 0.12 | 0.03 | 0.00 |
| | 1.20 | 1.29 | 1.40 | 1.56 | 1.82 | 2.20 | 2.79 | 3.70 | 5.03 |
| 80% band | 0.87 | 0.83 | 0.77 | 0.69 | 0.58 | 0.43 | 0.26 | 0.10 | 0.02 |
| | 1.13 | 1.18 | 1.25 | 1.34 | 1.48 | 1.68 | 1.95 | 2.30 | 2.71 |

Table 2 gives the limits for V_k/m for the 95 and 80 per cent. significance levels, and the bands are constructed so that the probability of an observed variance being above the upper limit equals the probability of its falling below the lower limit, and is 0.025 for the first case and 0.10 for the second. The independent variable in the table has been taken as n_k , the degrees of freedom, rather than k , in case grids of sizes other than 256 quadrats are used. Thus for a grid of 512 quadrats, $n_k = 256$ corresponds to $k = 1$, while for a grid of 256 quadrats, $n_k = 128$ corresponds to $k = 1$.

This table provides a test for randomness of greater scope and accuracy than Greig-Smith's (1952) relative variance test, in which V_1/m , which has expectation unity on the Poisson hypothesis, is tested against an approximate standard error $\sqrt{(2/n_1)}$. Examination of the table shows how large is the variation possible for the larger block sizes, so that quite large peaks at these points might easily be due to chance and not to any real ecological effect. This is due to the small number of degrees of freedom for these terms, but it should not be thought that because of this the extra calculation needed for a grid of 256 quadrats is wasted; the grid should be of this size in order that the variances for the smaller block sizes should

have available for them a large number of degrees of freedom. The chance variation in these terms is then much smaller than if the grid consisted of say only 64 quadrats.

(c) *Random Plants in a Mosaic of Phases*

This type of model attempts to represent a community in an advanced stage of development, different areas containing plants at different stages in a cycle of phases. Within each area every plant is independent of every other plant, but the density differs from area to area. In later models the non-randomness is of a much more complicated nature, the assumption of randomness within a phase or group being insufficient in most cases. However, it is useful to discuss it in order to test

TABLE 3
ANALYSES OF VARIANCE FOR MOSAIC EXPERIMENTS (GREIG-SMITH 1952)

| <i>k</i> | Experiment 4 | | Experiment 5 | | Experiment 6 | |
|------------|--------------|----------|--------------|----------|--------------|----------|
| | V_k | $E(V_k)$ | V_k | $E(V_k)$ | V_k | $E(V_k)$ |
| 1 | 0.19 | 0.24 | 0.23 | 0.23 | 0.39 | 0.44 |
| 2 | 0.19 | 0.24 | 0.21 | 0.23 | 0.38 | 0.44 |
| 4 | 0.25 | 0.24 | 0.26 | 0.25 | 0.40 | 0.50 |
| 8 | 0.30 | 0.24 | 0.31 | 0.24 | 0.47 | 0.53 |
| 16 | 0.16 | 0.26 | 0.28 | 0.45 | 1.01 | 0.76 |
| 32 | 0.15 | 0.25 | 0.26 | 0.31 | 1.04 | 1.01 |
| 64 | 0.07 | 0.30 | 0.82 | 0.81 | 0.57 | 0.78 |
| 128 | 0.07 | 0.30 | 0.01 | 0.77 | 1.36 | 0.83 |
| 256 | 0.03 | 0.24 | 0.03 | 0.25 | 0.02 | 0.43 |
| <i>m</i> | 0.24 | | 0.23 | | 0.43 | |
| m_H, m_L | 0.29, 0.20 | | 0.39, 0.10 | | 0.68, 0.10 | |

the (1952) conclusions of Greig-Smith, who based three sampling experiments on it, two (his experiments 4 and 5) with large, irregularly shaped areas and one (experiment 6) with smaller and more regular units. As with most of the models in this paper there are innumerable different cases possible if we vary lay-outs and density differences, so we confine discussion to Greig-Smith's actual examples, for which the lay-outs are given diagrammatically, and argue from the particular to the general using the theoretical results deduced.

In any mosaic model, with the plants distributed at random at two densities, the expected variances may be written in the form

$$E(V_k) = m + c_k(m_H - m_L)^2,$$

where m is the general mean, m_H, m_L are the mean numbers of plants per quadrat over the high- and low-density areas respectively, and c_k is a constant (for a given k) depending on the configuration of the mosaic. With more than two densities the

same equation will hold, assuming m_H and m_L represent the highest and lowest densities. Intermediate densities can easily be represented as a function of these two, and for a given configuration only c_k will change, according to the densities present. If $m_H = m_L$ the plants are all distributed with the same mean m and we have the result of Section II(b). Because of the method of calculation of the analysis of variance, blocks which contain plants at the same density, i.e. which fall completely inside one area of the mosaic, contribute nothing to the appropriate variance other than a term due to pure randomness. Thus it is only when the block size approaches that of the mosaic unit that there are any really large contributions to the variance; up to this critical block size we get small contributions from edge effects between blocks and mosaic units, and beyond it the behaviour of the variances will depend on the arrangement of the units; they may remain fairly steady or oscillate violently. In Table 3 we give the expected analyses of variance ($E(V_k)$) for the three cases (total discs only) using the same values as Greig-Smith did, together with the results of his experiments (V_k) and the various densities.

In experiment 4 the difference in density between high and low (0.09) was too small for any non-random effect to be detectable. The other two show the characteristic rise at block sizes corresponding to the size of the mosaic units, namely, between 64 and 128 quadrats in experiment 5 and between 16 and 32 quadrats in experiment 6. Greig-Smith (1952) defined two statistics which he used to summarize the information relating to clumped distributions. They were the *mean area of clump* (m.a.c.), a measure of the spread of the members of a group and defined as the area occupied by the individuals of a clump, and the *single clump area* (s.c.a.), the area containing on the average one clump, or the reciprocal of the mean density of clumps. If we regard a unit of the mosaic as a clump, then it is the m.a.c. which affects the analysis, and is the approximate point at which the rise in variance takes place. In Table 3, of course, the s.c.a. equals the m.a.c., but it is easy to see that if we considered only one set of discs (high density, say) the expected variances would only be altered by a common factor, as the coefficients c_k are unaltered; in fact the rise at the m.a.c. might be more obvious because m_L would be zero in the equation. Thus Greig-Smith's conclusions for this type of model are substantially correct. It is not certain that the peak will occur at the m.a.c., as subsequent terms may theoretically be higher, but generally the juxtaposition of areas of different density will mean that the densities for higher block sizes will tend to even out and the variances to be lower. This explains why the variances for the largest block sizes in experiments 5 and 6 are so small. The significance bands for the Poisson distribution can be used to advantage here to detect aberrant terms. In experiment 5, $E(V_{64})$ is outside the 95 per cent. band and $E(V_{128})$ just inside, while in experiment 6, $E(V_{16})$ is just inside and $E(V_{32})$ just outside. In the experimental results, V_{64} is significant in the first case, and both V_{16} and V_{32} are significant in the second.

(d) *Overdispersion of Individuals or Clumps*

A further stage in the development of a community is reached if the initial invaders of an area, assumed to be distributed at random, are allowed to become the parents of a generation of offspring plants. Greig-Smith (1952) used a similar

model (his experiments 2 and 3) in which the offspring are distributed at random inside a circle with the parent as centre, such that the area of each clump is just over three quadrats. This results (Thompson 1954) in a Neyman type A contagious distribution for the number of offspring in a single quadrat. Here, because of mathematical considerations, we let the offspring be distributed independently round the parent with their distances from it following a two-dimensional normal distribution. The resulting distribution of plants approximates to the negative binomial form if the numbers of offspring per group follow a geometric distribution. It seems more reasonable to suppose that the number of surviving offspring (as distinct

TABLE 4
 $E(V_k/m)$ FOR CLUMPED MODEL WITH RANDOM CENTRES

| $k:$ m.a.c. | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 |
|--|------|------|------|------|------|------|------|------|
| (a) <i>Offspring Alone</i> ($\lambda = 3$) | | | | | | | | |
| 9.4 | 1.12 | 1.19 | 1.64 | 1.84 | 2.40 | 2.58 | 2.91 | 3.02 |
| 4.7 | 1.31 | 1.46 | 2.04 | 2.24 | 2.69 | 2.83 | 3.08 | 3.16 |
| 2.4 | 1.64 | 1.84 | 2.40 | 2.58 | 2.91 | 3.02 | 3.19 | 3.25 |
| 1.2 | 2.04 | 2.24 | 2.69 | 2.83 | 3.08 | 3.16 | 3.28 | 3.33 |
| (b) <i>Parents and Offspring</i> ($\lambda = 3$) | | | | | | | | |
| 9.4 | 1.18 | 1.26 | 1.76 | 1.96 | 2.49 | 2.65 | 2.96 | 3.06 |
| 4.7 | 1.41 | 1.57 | 2.15 | 2.34 | 2.76 | 2.89 | 3.11 | 3.19 |
| (c) <i>Offspring Alone</i> ($\lambda = 6$) | | | | | | | | |
| 9.4 | 1.26 | 1.43 | 2.40 | 2.84 | 4.09 | 4.47 | 5.21 | 5.44 |
| 4.7 | 1.68 | 2.00 | 3.28 | 3.73 | 4.72 | 5.03 | 5.57 | 5.74 |

from seeds) will be limited for ecological reasons, so we have taken the distribution of offspring per group as binomial, with a mean of 3 (Thompson 1955). This gives a degree of contagion less than the negative binomial distribution but greater than the Neyman type A distribution, and it will be fairly safe to assume that the results to be expected from Greig-Smith's model will lie somewhere between the results of this section and Section II(b).

With the two-dimensional normal distribution the extent or spread of a single clump depends on the value of the parameter σ in the function $e^{-r^2/2\sigma^2}/(2\pi\sigma^2)$. We can specify an area containing on the average 95 per cent., say, of the distribution and call it arbitrarily the m.a.c. We do not consider clumps above about 10 quadrats in size since they would then be so diffuse as to have hardly any meaning and the possibility of several clumps overlapping would be much greater. Table 4 gives the expect-

ed variances, adjusted to $m = 1$, for four different values of the m.a.c. and with either the offspring alone present (assuming the parents to have died off) or parents and offspring together. Two only of this latter case are given to show how small are the differences. Note that m or its sample value \hat{m} indicates throughout the paper the mean per quadrat of all classes of plants actually present, and not merely the original random parents. If it is required to find the expected variances $E(V_k'/m)$ for this type of model when the mean number of offspring per group λ is different from 3, it will be sufficiently accurate to use the formula

$$E(V_k'/m) = 1 + \frac{2\lambda-1}{5} \{E(V_k/m)-1\},$$

where the values of $E(V_k/m)$ are read from Table 4 ((a) or (b)). This applies when the distribution of offspring is binomial, and two illustrative examples are given in Table 4(c) with $\lambda = 6$. If the numbers of offspring are to follow a Poisson series with mean λ , then the appropriate adjustment formula is

$$E(V_k'/m) = 1 + 0.4\lambda\{E(V_k/m)-1\}.$$

The first point to notice about these results is that all variances are expected to be greater than the mean, a consequence of the overdispersion of individuals. This, and the fact that $E(V_k)$ increases gradually with k to a limit (whose value depends only on the distribution of the offspring, and is $m(\lambda + \frac{1}{2})$ if they are binomial, $m(\lambda + 1)$ if they are Poisson) are the two features which combine to identify a clumped distribution with the clumps distributed at random. The shape of the variance-block-size graph is virtually the same for all combinations of the m.a.c. and λ , except for the general level of the graph and its rate of rise. These two variables, the m.a.c. and λ , are intimately related in their effect on the shape of the graph, but some contrasting cases can nevertheless be distinguished. Generally speaking, the steeper the rate of rise is the higher λ is, and the nearer the corrected relative variance V_1/m is to unity (for a given λ) the higher is the m.a.c. There are two limiting cases: as the m.a.c. becomes very small, so that the offspring are all in the same quadrat as their parent, $E(V_k)$ tends towards the limits given above for all k ; while if the m.a.c. is very large, so that a single clump is diffused over many quadrats and the divisions between clumps are non-existent, we have virtually the Poisson case of Section II(b), $E(V_k)$ tending to m for all k .

In his experiments 2 and 3 Greig-Smith (1952) tried to relate what must have been chance peaks in his graphs to either the m.a.c. or the s.c.a. It is quite evident that the s.c.a. can have no effect other than on the general level of the graph (through m), since the clumps are completely independent. We have seen that the m.a.c. can have a marked effect on the graph, but there is unfortunately no outstanding feature of the theoretical results from which we could deduce a method for determining it exactly. The only obvious conclusions, from an examination of Table 4 and Figure 2, where some of the results from the table are presented graphically, are that it occurs either at or just before the biggest single jump between adjacent values, and that the value of the variance at the m.a.c. is in every case a little below half way between the theoretical lower and upper limits. It is doubtful whether points such as these would be of any value in practice, especially as the

sampling variation is so much larger in this non-random case than for the simple Poisson distribution. It is just possible that, with a function which gives a definite limited area of spread of the offspring in a group, the curve might show a more marked upward trend at the m.a.c. than with the two-dimensional normal distribution, which has a long "tail".

The extensions of the simple model, retaining randomly distributed clumps, are innumerable, and in every case exactly the same features are displayed in the analysis of variance. The most obvious generalization is to let each offspring become the parent of another generation of plants, which follow the same laws governing numbers and distances as the previous generation. This process may be continued

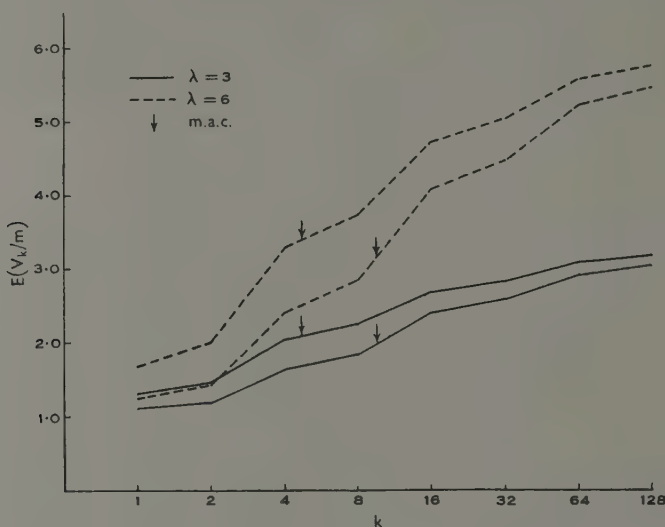


Fig. 2.—Expected variances, plotted against block size (data of Table 4).

to any stage, say the g th generation, and we can consider all g generations or any consecutive set to be present simultaneously, thus allowing for the dying out of earlier generations. In the latter case we have effectively overdispersion of clumps as well as of individuals, for the overdispersed individuals of the basic model have become the centres of distribution here.

(e) Underdispersion of Clumps

So far we have ignored the effects of competition on the spread of plants. The models discussed in Section II(d) have been most likely to apply to the pioneer or building phases of a community when there are few centres, whose distribution may effectively be taken as random. As soon as the density increases beyond a certain point the offspring from different groups must compete with each other for the available space, and this will tend to produce underdispersion of clumps, which means that the clumps themselves will be distributed more evenly than if they were at random. Thus the s.c.a. should have some meaning, since each clump

will occupy a well-defined area more or less separate from other clumps. The model constructed to illustrate underdispersion of clumps is a highly idealized one, but it nevertheless provides a guide to the expected behaviour in more complicated

TABLE 5
 $E(V_k/m)$ FOR OFFSPRING OF UNDERDISPERSED CENTRES
m.a.c. = 4.7, $\lambda = 3$

| $k:$ d | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 |
|-------------|------|------|------|------|------|------|------|------|
| 4 | 1.31 | 1.45 | 1.74 | 2.07 | 0.66 | 0.63 | 0.58 | 0.57 |
| 3 | 1.28 | 1.42 | 1.05 | 1.23 | 0.90 | 1.10 | 0.82 | 1.03 |
| 2 | 1.05 | 1.12 | 0.79 | 0.75 | 0.66 | 0.63 | 0.58 | 0.57 |
| 4* | 1.68 | 2.00 | 2.68 | 3.38 | 0.66 | 0.64 | 0.58 | 0.57 |

* Mosaic of clumps at two densities, $\lambda = 3, 9$.

practical situations. The clumps are assumed to be distributed such that the centres lie on the intersections of a square lattice superimposed on the grid. Each centre is d units of length away from its nearest neighbours on the lattice (the side of a quadrat being one unit) so that the s.c.a. is d^2 quadrats. This representation can perhaps be considered as an expected situation when a community has reached

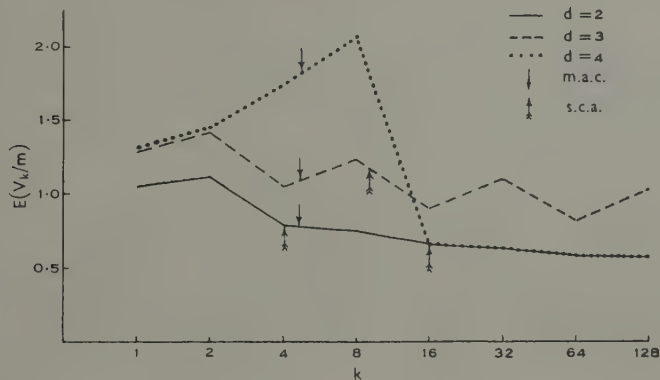


Fig. 3.—Expected variances, plotted against block size (data of Table 5).

a mature stage in its cycle of development and the previous generations have died out. In any actual sample clumps would not be so evenly spaced, of course, but the average distance between clumps would tend to be of the order of d . The distribution of offspring is such that the m.a.c. is 4.7 quadrats and $\lambda = 3$, as in Table 4(a). The results for three different values of d are presented in Table 5 and Figure 3, and it is simple to deduce from these what would occur if other values of the m.a.c. and d were taken.

A real effect due to underdispersion of clumps is noticeable only when d^2 is appreciably larger than the m.a.c., as with $d = 4$. Then there is a rise in variance to the block size before d^2 ($k = 8$) and a sudden drop at the s.c.a. ($k = 16$). Otherwise there is no clearly defined drop, but a steady decrease after an initial rise. This initial rise is due to the clumping of offspring round the centres, and the effect up to the block size before d^2 is almost exactly the same as in the random centres case (Table 4). The m.a.c. has no effect on this type of distribution, except in so far as it is related to the s.c.a. in overcrowded communities, so that the second conclusion of Greig-Smith's (1952) summary is incorrect. The peak, if one is obvious, should occur just before the s.c.a. Similar results apply if the clumps are distributed with two or more different densities, as is shown in the last line of Table 5, where half the clumps have a mean of 3 plants and half a mean of 9, an appreciable difference in density. The essential difference between this model and that of Section II(c), where the peak occurred at the m.a.c., lies in the clumping of the plants. With randomly distributed plants the units of the mosaic are not really clumps at all since the plants are independent of each other. Something approximating to the model of this section seems more likely to occur in the field.

(f) *Underdispersion of Individuals*

The two-dimensional normal distribution, used in Section II(d) to obtain the distances between related plants of successive generations and leading to overdispersion of individuals, is not really suitable when we proceed beyond the second generation. This is because the spread may take place in any direction, and in practice its use would result in succeeding generations of offspring tending to spread inwards on spaces already occupied, as well as outwards. We would be more likely to find later offspring radiating out from the original centre, and this effect can be partly achieved by using the following function for the distances x along a line between successive plants:

$$f(x) = 4xe^{-2x/c}/c^2,$$

where c is a constant. This is a χ^2 distribution with 4 degrees of freedom, and a two-dimensional spread is obtained by combining two such independent functions along rectangular axes. In this way spread is limited to one quadrant of a circle, but a unidirectional effect is obtained, and the probability of having two plants too close together is small. The model was suggested by the example of Phillips (1954), in which a definite forward move is made in each generation owing to vegetative spreading of the plants in approximately the same direction. In the present artificial model we consider a chain of n plants generated in the above manner, with the original plant of each chain distributed at random over the grid. The constant c is considered in relation to the length of side of a quadrat; the average distance between successive plants along a side is c , and the probability that they will be not more than $2c$ apart is about 0.90. In Table 6 we have used two values of c which give reasonable values of the spread, and two values of n .

We note that the initial variances are less than m so that underdispersion results, and the effect becomes more pronounced as n increases or c decreases or both.

After the initial drop the later variances increase again owing to the randomness of the groups. This model can serve as a basis for more complicated models in which other plants are distributed round each plant of a chain. Thus each member of the chain would become the centre of distribution of a clump and something analogous to Phillips's (1954) example would be obtained. The clump size would bear some relation to the constant c , and it is most likely that it would be of the order of c^2 or $2c^2$, which from Table 6 is the block size just before the variance increases above the mean. A model along these lines has not yet been formulated because of the mathematical difficulties involved, but Phillips's results show what would happen in such a case, and we proceed now to examine them in the light of the theoretical experience gained.

TABLE 6
 $E(V_k/m)$ FOR RANDOM GROUPS OF UNDERDISPERSED PLANTS

| k : | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 |
|--|------|------|------|------|------|------|------|------|
| $n = 2 \begin{cases} c = 2 \\ c = 1 \end{cases}$ | 0.98 | 0.90 | 0.97 | 0.93 | 1.48 | 1.71 | 2.61 | 2.89 |
| | 0.93 | 0.81 | 1.27 | 1.42 | 2.21 | 2.45 | 3.00 | 3.17 |
| $n = 4 \begin{cases} c = 2 \\ c = 1 \end{cases}$ | 0.96 | 0.84 | 0.93 | 0.79 | 1.16 | 1.71 | 3.93 | 4.07 |
| | 0.89 | 0.61 | 1.28 | 1.24 | 3.27 | 3.85 | 6.29 | 6.90 |

III. EXAMINATION OF FIELD RESULTS

(a) *Data of Phillips (1954)*

Phillips originally gave (unpublished Ph.D. thesis, Manchester University, 1952) the analyses of variance for about 20 samples of 256 (usually) or 512 quadrats from *Eriophorum* communities. This is sufficient for us to determine how far her conclusions are in accord with theory. We give first a brief description of the morphology of *Eriophorum*, for this is an essential adjunct to an appreciation of the features to be expected in the analysis of variance. The plant spreads by means of rapidly growing rhizomes, up to 30 or more centimetres in length (the quadrat size used was 100 sq. cm). At the end of the rhizome a swollen stock appears bearing the leaves of a tufted aerial shoot, which is the plant proper and which occupies a very small area in relation to quadrat size. More than one rhizome can appear on a single stock; daughter rhizomes up to three in number may be formed from axillary buds, arising in positions equally spaced round the stock, so that if all occur the angles between them are 120 degrees. They can occur in either of two basic positions, one forward and two backward, or two forward and one backward, symmetrically placed in each case with respect to the direction of spreading. The forward rhizomes are always longer than the backward; average lengths are somewhat over 20 cm for forward and less than 10 cm for backward rhizomes. Groups of upwards of 30 shoots can thus arise from a single parent.

Such a system is eventually broken up by the death and decay of the oldest constituent shoots. This occurs when the original shoot flowers, after about four

or five generations are present in the group, so that in successive seasons the group separates into clumps of shoots consisting of three or four seasons' growth from a single parent. Phillips maintained that the analysis of variance on a 16×16 grid should detect two types of heterogeneity, neglecting any larger-scale heterogeneity relatable to differences in environment and frequently detectable by inspection. The clumping of shoots at the ends of forward rhizomes produces underdispersed clumps at distances of about 20 cm or more, and thus a s.c.a. of somewhere between 4 and 16 quadrats with a m.a.c. of about 4 quadrats or a little less. Secondly, the association of four or five generations of living shoots linked by rhizomes results in a larger-scale clump size of between 32 and 128 quadrats. From these morphological facts she asserted that a variance graph with a double peak should result, a primary one at about $k = 8$ due to the grouping of first generation daughter rhizomes round parent shoots, and a secondary one at about $k = 64$ due to the presence of several "single-plant clumps" (her term) of about four or five generations, each having arisen from separate parents. We tabulate below (Table 7) eight of

TABLE 7
TABLE OF V_k FOR DISTRIBUTIONS OF ERIOPHORUM

| Grid: k | 5B | 19 | 2A | 2B | 16 | 23 | 25 | 11B |
|--------------|------|------|-------|------|------|-------|------|------|
| 1 | 1.10 | 0.24 | 0.76 | 0.80 | 0.74 | 1.13 | 0.44 | 0.59 |
| 2 | 1.11 | 0.45 | 0.75 | 0.75 | 0.91 | 1.57 | 0.81 | 0.54 |
| 4 | 1.00 | 0.29 | 0.61 | 0.80 | 0.95 | 1.71 | 0.96 | 0.77 |
| 8 | 0.96 | 0.27 | 0.46 | 1.15 | 2.19 | 0.94 | 1.10 | 0.69 |
| 16 | 0.87 | 0.34 | 0.97 | 0.82 | 0.72 | 5.25 | 1.34 | 1.37 |
| 32 | 3.76 | 1.07 | 2.48 | 2.59 | 0.56 | 8.77 | 0.73 | 1.12 |
| 64 | 2.10 | 0.50 | 10.41 | 1.36 | 5.91 | 12.29 | 0.98 | 0.23 |
| 128 | 0.14 | 0.14 | 19.14 | 2.85 | 0.32 | 10.10 | 0.25 | 0.25 |
| \hat{m} | 1.09 | 0.34 | 0.77 | 0.80 | 0.95 | 1.39 | 0.51 | 0.62 |

the analyses from the unpublished thesis (mostly reproduced in the 1954 paper), selected to give as representative a coverage as possible of her results.

Phillips, following Greig-Smith (1952), assumed that a peak indicated either the approximate m.a.c. or a unit in a mosaic of different densities. The latter statement might conceivably be true if the plants are randomly distributed in the phases (cf. Table 3), but generally it is the block size after the peak which is the significant one in the case of underdispersed clumps (cf. Table 5), and the occurrence of a peak naturally presupposes a drop in the term after it. From the theoretical results we should expect V_1 to be of the order of \hat{m} or less because of the local underdispersion of individuals produced by the rhizome spreading process (analogous to that in Section II(f)), and this is so from Table 7. Since there is a measure of clumping (of daughter rhizomes round parent shoots) and these clumps are more or less regularly spaced, we have an approximation to the model of Section II(e),

though without the extreme overdispersion of individuals induced by the distance functions used there. If the s.c.a., estimated at between 4 and 16 quadrats, is actually greater than the m.a.c. (at most 4 quadrats), the characteristic rise for the clumped distributions will appear as in Table 5 for $d = 4$ up till the block size before the s.c.a., followed by a drop at this block size. Otherwise, if the two are nearly equal, the rise may not appear or not be obvious (cf. Table 5, $d = 2$). The association of these small clumps into "single-plant clumps", of areas between 32 and 128 quadrats, means that the higher block sizes will be affected in a similar or the same way as the small clumps affect the small block sizes. The variance should rise again after about $k = 16$ and, if the single-plant clumps are randomly distributed, should be maintained for all higher k . If they are underdispersed, owing to competition say, then there will be another fall in variance, perhaps not until $k = 64$ or 128, in which case it could easily be due to chance.

The chance factor has not really been taken into account by Phillips; most of her samples, however, show this fall and its repeated appearance may be sufficient justification for the conclusion. She does not make enough of the fact of randomness of single-plant clumps when the variance is maintained, as in grids 2A, 2B, and 23 of Table 7, often attributing it rather to "differences in density between the two halves of the grid". This, and the fact that she confuses the peak of a graph with the appropriate clump size, are her only major errors. She relates a very small primary peak, as in grid 5B, to a reduction in the number of daughter rhizomes per parent shoot, a correct conclusion on the basis of Table 4. The initial effects in grids 2A, 2B, and 11B may also be due to this effect in a more extreme way, for they are similar to the expected variances with the model of Section II(f), where there are no offspring plants. Grids 16 and 25 also show marked underdispersion at first, but the long subsequent rise and a higher than usual s.c.a. between 16 and 32 quadrats would possibly indicate that the rhizomes are longer than average here and the m.a.c. therefore larger. In neither of these cases, however, are the troughs significantly low, and it is only the experience gained from all the other grids that enables these conclusions to be drawn. The reverse effect, due to short rhizomes, is shown in grids 19, 2A, and 23 by the small values of the s.c.a. It might be argued that Phillips's conclusions on the primary peaks (due to the small clumps) are unsound, as they are based on very small rises in variance and the terms would often be well within the limits of error arrived at by using the significance band approach. However (and this is one justification for the use of the method), she has so many samples all showing the same trend that it cannot always be a chance effect.

(b) *Data of Cain and Evans (1952)*

This consists of the fully mapped distributions, in contiguous quadrats of 1 sq. metre over an area of 7640 sq. metres, of the individuals of three species of herbs, *Lespedeza capitata* Michx. (bush clover), *Liatis squarrosa* Willd.* (blazing star), and *Solidago rigida* L. (goldenrod). In each species a tendency of the plants to

* Referred to by Cain and Evans as *L. aspera*.

occur as clumps was readily apparent, most of all in *Lespedeza* and least in *Solidago*; we exclude the intermediate case, in which the density of plants was very low, only 0.04 per quadrat, and concentrate on these two. The authors distinguished between "major" clumps (large concentrations of closely spaced individuals) and

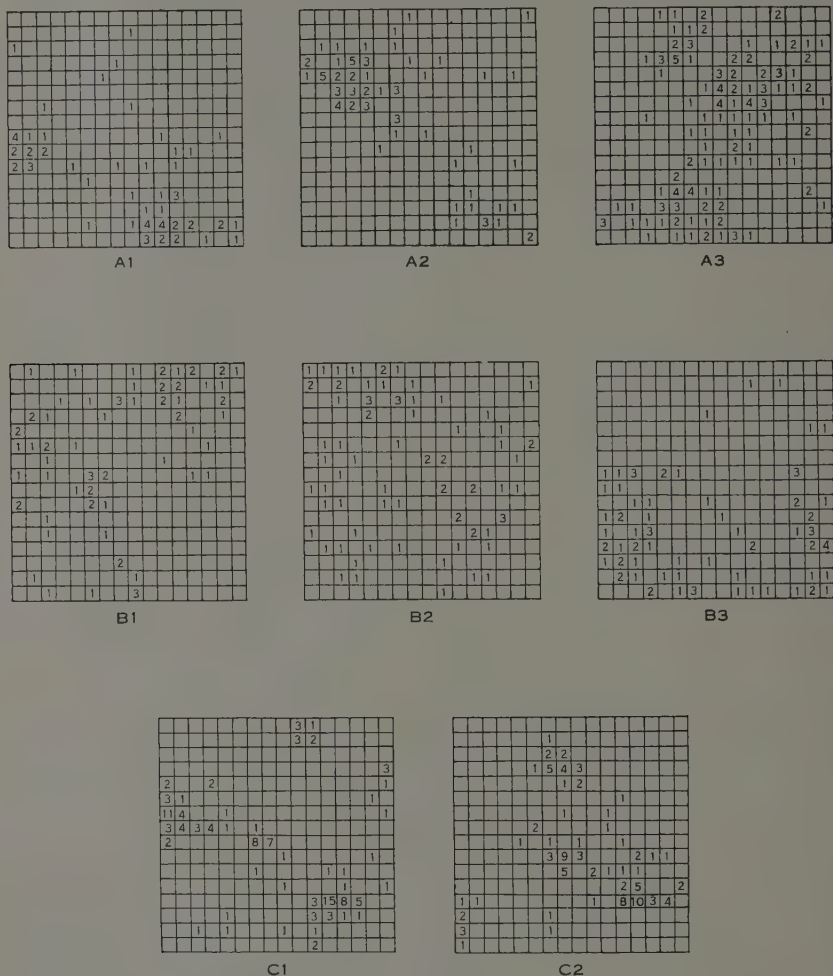


Fig. 4.—Counts of plants in grids superimposed on distribution maps (Cain and Evans 1952). A, *Lespedeza* (1 sq. m quadrats). B, *Solidago* (4 sq. m). C, *Lespedeza* (4 sq. m).

"minor" clumps (little groups of two, three, or four individuals each). In the distribution of *Lespedeza* there were several major clumps, occupying areas from 8 to 32 quadrats, as well as scattered individuals and minor clumps, so that there was very pronounced overdispersion, due, from the available evidence, to the heavy nature of the seeds and consequent localized dispersion of offspring round parents. From inspection the distribution pattern looks similar to the models of Section

II(d). The *Solidago* distribution showed a strong development of minor clumps and scattered individuals, and overdispersion, as measured by the ratio variance : mean, was still present. This plant possesses a shallow creeping rhizome by which it propagates itself, as well as the seed mechanism, the seeds being light in comparison with those of *Lespedeza*. The authors considered the rhizome spread to be responsible for the minor clumping, and the closer approach to randomness due to the role that chance might be expected to play in the more widespread distribution of seeds by wind. The rhizome spread suggests a similarity to the model of Section II(f), but the presence of seed dispersal as well might complicate matters. If it is indeed widespread, the lower block sizes might show the local rhizome dispersal trend alone (compare the model of Section II(d) with a very large m.a.c.).

Figure 4 gives the detailed counts of the plants in the quadrats of grids selected from the distribution of each species as plotted on the published maps, and the

TABLE 8
TABLE OF V_k FOR THE GRIDS IN FIGURE 4

| Grid: k | A1 | A2 | A3 | B1 | B2 | B3 | C1 | C2 |
|--------------|--------------|-------------|-------------|------|------|-------|-------------|-------------|
| 1 | 0.26 | 0.42 | 0.61 | 0.38 | 0.37 | 0.48 | 1.08 | 1.11 |
| 2 | 0.24 | 0.67 | 0.69 | 0.24 | 0.28 | 0.30 | 2.50 | 1.91 |
| 4 | 0.34 | 0.37 | 0.70 | 0.48 | 0.09 | 0.37 | 5.11 | 2.06 |
| 8 | 0.93* | 0.98 | 0.96 | 0.48 | 0.80 | 0.43 | 5.35 | 2.54 |
| 16 | 2.06 | 0.43 | 5.77 | 0.27 | 0.73 | 1.52 | 6.02 | 2.88 |
| 32 | 4.05 | 1.40 | 7.18 | 1.95 | 1.12 | 0.73 | 12.63 | 0.84 |
| 64 | 1.14 | 9.77 | 4.78 | 0.37 | 1.33 | 0.27 | 5.50 | 2.04 |
| 128 | 10.97 | 6.25 | 1.27 | 5.06 | 0.25 | 11.39 | 1.13 | 10.16 |
| \hat{m} | 0.25 | 0.30 | 0.60 | 0.30 | 0.31 | 0.34 | 0.50 | 0.41 |

* Values at approximate m.a.c. for *Lespedeza* shown in bold face type.

corresponding analyses of variance are given in Table 8. Selection rather than random choice was necessary because of the large more or less empty spaces in each distribution. In the *Lespedeza* case (grids A1, A2, and A3), coverage of at least one or two major clumps was attempted. For *Solidago* (grids B1, B2, and B3), quadrats were grouped in square blocks of four so that the quadrat size was 4 sq. metres, because the density was so low that 1 sq. metre quadrats gave too few numbers in a grid to be of any use. Therefore grids C1 and C2 with 4 sq. metre quadrats were taken from the *Lespedeza* map to compare the use of different basic quadrat sizes.

Analysing the *Lespedeza* results first, we note the good agreement with the hypothesis of random centres and clumped offspring. In all five grids V_1 is greater than \hat{m} , indicating overdispersion (extreme in grids C1 and C2) and there is a rise

to high variances for large k . The effect of using 4 sq. metre basic quadrats has been to shift the rise to lower block sizes, as expected, and it is notable that in every case the rise corresponds to a block size nearly at the m.a.c. (estimated for each grid from the maps and inserted in Table 8). Thus the conclusion drawn in Section II(d) on this effect may be a reasonable one on the basis of this evidence. A dispersal mechanism with a much shorter "tail" to its frequency distribution than the two-dimensional normal distribution is indicated here, so that the m.a.c. is much more clearly defined. The high values of the variances beyond the m.a.c. suggest that there are several generations present in the larger clumps and that the spread per generation is not of a very high order.

In all three *Solidago* grids (B1, B2, and B3) the values of V_1 indicate overdispersion of individuals in accordance with the results of Cain and Evans for the whole distribution. Therefore there is some clumping of individuals, even if only slight. We can make use of the significance band method here to test if the distri-

TABLE 9
 V_k/\hat{m} FOR THE SOLIDAGO GRIDS, COMPARED WITH 95% SIGNIFICANCE BAND FROM TABLE 2

| k : | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 |
|----------|------------|------------|------------|------------|------------|------------|------------|------------|
| Grid B1 | 1.3 | 0.8 | 1.6 | 1.6 | 0.9 | 6.5 | 1.2 | 16.9 |
| Grid B2 | 1.2 | 0.9 | 0.0 | 2.6 | 2.4 | 3.6 | 4.3 | 0.8 |
| Grid B3 | 1.4 | 0.9 | 1.1 | 1.3 | 4.5 | 2.1 | 0.8 | 33.5 |
| 95% band | 0.7 1.3 | 0.7 1.4 | 0.6 1.6 | 0.4 1.8 | 0.3 2.2 | 0.1 2.8 | 0.0 3.7 | 0.0 5.0 |

butions conform to the hypothesis of randomness. Adjusting the variances for grids B1, B2, and B3 so that $\hat{m} = 1$, we compare them with the 95 per cent. band for the Poisson distribution taken from Table 2. This is done in Table 9, and the values which appear significantly high shown in bold face type.

There are too many significantly high variances for the distributions to be regarded as random, and the general trend seems to be for high variances to be maintained for large k , suggesting clumped distributions. In every case V_2 is appreciably less than V_1 , and in addition V_4 in grid B2 continues this trend; it is suggested this shows an underdispersion of clumps of size 2 quadrats, i.e. 8 sq. metres, possibly due to the rhizome spread. The subsequent rise in variance is considered to be due to the more widespread seed dispersal which causes groups of such small clumps to be random or overdispersed, the former being more likely because of the low density of plants. A simple examination of the grids themselves would hardly give the information that has been deduced from the analyses of variance in this example, and this is a strong point in favour of the method when the distribution pattern does not display any obvious features.

IV. DISCUSSION AND CONCLUSIONS

We proceed now to attempt some evaluation of the grid method and determine whether it is of any value as a practical technique. The large sampling errors inherent in the processes expose one real weakness of the method. The virtual independence of the V_k for large k means that a variety of variance graphs is possible (within given probability limits) under ordinary random sampling of a model, each of which could easily correspond to the expected graph of widely different models. In the two cases of randomly distributed plants in a mosaic of phases (Section II(c)) and clumped offspring round random centres (Section II(d)) the variance graphs have similar shapes and confusion could arise. If then we are given an analysis of variance for which the model generating the distribution is unknown, we could not with any degree of reliability determine the nature of the model except possibly in rare cases. A knowledge of the chance variation possible would have influenced Greig-Smith (1952) to make rather different decisions sometimes. It might be argued that conclusions have been drawn in the practical examples from results which do not appear very significant statistically. Such are the conclusions on small clumps from the data on *Solidago* (Table 9) and in Phillips's (1954) data (Table 7), where small rises and falls in variance have been made the basis of decisions on clumping effects. The main point about these trends in variance is that, though small, they appear in all or nearly all of the analyses in both cases, and we can place more reliability on such results than on perhaps a single significant one. This points the way to an effective use of the grid method in the field: provided repeated sampling of a population or community is undertaken it should be possible to distinguish chance effects from the steady trends inherent in the model describing the community.

In most field work on the ecology of a species or group of species forming a plant community, ecologists also study the morphology of the plants under observation. The significance of a particular pattern of distribution can be understood only if the factors and processes that have produced it are known. Cain and Evans (1952) acknowledge the existence of several factors affecting the local distribution of a plant species, including: (1) the microclimate; (2) the biology of the species, especially the reproduction and dispersal mechanisms; (3) the relations to other species in the community; (4) the element of chance in the dispersal and establishment of new individuals. Any description of a distribution pattern will be inadequate and lack meaning unless it can be interpreted in terms of the processes involved. For our purposes the most important of these points is (2). It means that a satisfactory practical investigation must include a study of the essential factors generating the distributional pattern. These being given, we should be able to classify the process into one or a combination of the basic theoretical models described in Section II, and so have a foreknowledge of the expected analysis of variance. This may show distinct features which would be immediately recognizable without a prior knowledge of the model; but it is perhaps more likely, as in the field examples of Section III, that the trends are of small magnitude and the expected values of the terms showing a trend are inside the significance band for some other model. Nevertheless we know this trend to exist, and appropriate conclusions can be drawn

from its presence, or absence, or magnitude, etc. in relation to other ecological factors. Thus we have achieved one of the purposes of carrying out an ecological analysis, and have provided a justification for the use of a grid of quadrats.

In conclusion it must be emphasized that the approach in this paper to the problem of determining the distributional pattern is an empirical one, in that no attempt has been made to estimate statistically the parameters of a model such as those relating to the distributions of distances and numbers of offspring in clumps. Apart from the fact that this is a very difficult problem, we could not be sure in any field sample that the correct functions specifying these distributions had been chosen, and further the sort of sampling variation encountered in these processes would most probably prevent the attainment of any accuracy in estimation. Rather must we confine ourselves to simpler methods of estimation, of more general effects such as sizes of clumps or average number of plants per clump, effects determinable approximately from the variance graph. These are the effects which interest the ecologist most, and it is suggested that a proper application of the grid sampling method will provide this information.

V. REFERENCES

- ASHBY, E. (1948).—Statistical ecology. II. A reassessment. *Bot. Rev.* **14**: 22.
- CAIN, S. A., and EVANS, F. C. (1952).—The distribution patterns of three plant species in an old-field community in southeastern Michigan. *Contrib. Lab. Vertebr. Biol. Univ. Mich.* No. 52.
- CLAPHAM, A. R. (1936).—Overdispersion in grassland communities and the use of statistical methods in plant ecology. *J. Ecol.* **24**: 232.
- GREIG-SMITH, P. (1952).—The use of random and contiguous quadrats in the study of the structure of plant communities. *Ann. Bot., Lond. (N.S.)* **16**: 293.
- PHILLIPS, M. E. (1954).—Studies in the quantitative morphology and ecology of *Eriophorum angustifolium* Roth. II. Competition and dispersion. *J. Ecol.* **42**: 187.
- THOMPSON, H. R. (1954).—A note on contagious distributions. *Biometrika* **41**: 268.
- THOMPSON, H. R. (1955).—Spatial point processes, with applications to ecology. *Biometrika* **42**: 102.

STUDIES ON THE ORIGIN, EVOLUTION, AND DISTRIBUTION OF THE GRAMINEAE

II. THE TRIBE PANICEAE

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Summary

A world distribution map of the tribe Paniceae has been prepared, based on the percentage frequency of species of Paniceae in the total grass flora of each region. The map shows that relative specific differentiation is higher in the western hemisphere than in the eastern hemisphere, and that there is a zone of maximum concentration in north-eastern South America. The significance of this distribution pattern is discussed, and it is shown that the taxonomic evidence does not support a New World origin for the tribe.

The distribution of the Paniceae in the United States shows a close relationship to winter temperature, and especially to annual rainfall. These climatic factors are also of major importance in other parts of the world, and in large measure explain the present distribution of the tribe.

The Paniceae and Andropogoneae both appear to be "natural" tribes, which probably originated from a common panicoid stock in the warmer parts of the eastern hemisphere, possibly in the East Africa-Madagascar region. The Paniceae spread rapidly throughout the tropics and subtropics of both hemispheres, especially in the equatorial zone with high temperatures and well-distributed rainfall. They show a pattern of climatic adaptation differing from that of the Andropogoneae. The latter, apparently formed more recently from the panicoid stock, reach their highest development in monsoonal climates with a much shorter season of heavy rainfall.

I. INTRODUCTION

This paper, dealing with the tribe Paniceae, is the second in a series dealing with the present distribution of the tribes of grasses, as influenced by climatic and other factors. The first paper in the series dealt with the tribe Andropogoneae (Hartley 1958). The studies of individual tribes are extensions of a preliminary paper (Hartley 1950) which drew attention to the close relationship between climatic factors and grass distribution.

II. THE TRIBE PANICEAE

The tribe Paniceae, which with more than 1460 species is the largest of the tribes of the Gramineae, has been accepted as a natural one ever since it was originally established early in the nineteenth century. However, there have been differences of opinion between various authorities on the precise limits of the tribe,

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TABLE 1
PANICEAE AND APPLIED TRIBES ACCORDING TO LEADING AUTHORITIES

| | | | | | | | |
|-----|----------------|----------------|-----------|----------|--------------|--------------|--------------|
| 1 * | Paniceae | | | | | | |
| 2 | Paniceae | | | | | | |
| 3 | Tristegineae | Paniceae | | | | | |
| 4 | Tristegineae | Paniceae | | | | | |
| 5 | Melinideae | Paniceae | | | | | Boivinelleae |
| 6 | Tristegineae | Paniceae | | | | | |
| 7 | Paniceae | | | | | | Boivinelleae |
| 8 | Melinideae | Paniceae | | | | | |
| 9 | Arthropogoneae | | Paniceae | | | | |
| 10 | Melinideae | Arthropogoneae | Isachneae | Paniceae | Anthephoreae | Lecomtelleae | Trachyeae |
| 11 | Melinideae | Arthropogoneae | Isachneae | Paniceae | Anthephoreae | Lecomtelleae | Trachyeae |

* References: 1, Kunth 1833. 2, Steudel 1855. 3, Benthams and Hooker 1883. 4, Hackel 1887. 5, Bews 1929. 6, Avdulov 1931. 7, Hubbard 1934. 8, Hitchcock 1935. 9, Pilger 1940. 10, Pilger 1954. 11, Tateoka 1957.

and on the genera to be included in it. This is illustrated in Table 1, based partly on Avdulov (1931), in which the concepts of leading authorities are shown schematically.

The earlier authors adopted a broad view of the tribe, including in it substantially all the genera then known which have at any time been regarded as coming within its limits. Subsequently, Bentham and Hooker, followed by Hackel and more recently by Avdulov, separated some of the genera to constitute the tribe Tristegineae. This was a somewhat heterogeneous group, which included not only most of the genera which have been placed in the Melinideae and Arthropogoneae by later authors, but also some more distantly related genera which are now commonly placed in the Agrostae and other tribes. Partly because of the lack of clear characterization of the Tristegineae, leading agrostologists in England, America, and Germany reverted to a broad concept of the Paniceae, maintaining separate only the Boivinelleae (Hubbard), the Melinideae (Hitchcock), and the Arthropogoneae (Pilger 1940). Related groups of genera within the Paniceae were treated as subtribes, but in the most recent classifications, represented by those of Pilger (1954) and Tateoka (1957), these subtribes have been raised to the status of independent tribes. Thus the genera which had been included in the Paniceae *sensu lat.* are now placed in eight separate tribes.

However, the schematic presentation in Table 1 gives a somewhat false impression of the importance of these differences, for more than 80 per cent. of the genera and 90 per cent. of the species remain within the Paniceae even when the tribal limits are restricted. Most of the other tribes are very small, consisting of one or a few, often monospecific, genera. This is shown by the following figures, taken from Pilger (1940) and Potztal (1956):

| Tribe | Genera | Species | Tribe | Genera | Species |
|--------------|--------|---------|----------------|--------|---------|
| Paniceae | 82 | >1460 | Boivinelleae | 3 | 4 |
| Melinideae | 3 | 64 | Lecomtelleae | 1 | 1 |
| Isachneae | 4 | c. 66 | Trachyeae | 1 | 1 |
| Anthephoreae | 1 | c. 20 | Anthropogoneae | 4 | 11 |

This numerical predominance of the Paniceae *sensu str.* involves the consequence that the pattern of geographical distribution, discussed later in this paper, is not greatly affected by the varying concepts of tribal limits.

III. THE DISTRIBUTION MAP

A world distribution map of the Paniceae, prepared on a similar basis to that previously used in mapping the Andropogoneae, is shown in Figure 1. For a description of the method of mapping and the criteria for the selection of the floras and regional lists used, reference may be made to paper I in this series (Hartley 1958). A broad concept of the tribe has been adopted in analysing floras for the purpose of this mapping, corresponding to that of Hubbard (1934) as shown in Table 1.

Seven zones are shown on the map, with percentages of species of Paniceae in the total grass flora of <10, 10-20, 20-30, 30-40, 40-50, 50-60, and >60 per cent. respectively. Partly because of the greater number of lines shown, the pattern

is somewhat more complex than that of the distribution of the Andropogoneae, but it is nevertheless basically well defined and coherent. Only six of the more than 320 floras and regional lists used in the preparation of the map do not fit into the pattern as shown. These "anomalies", shown by crosses on the map, are all in regions affected by special local influences. They include the Nile Delta and Nile Valley of Egypt, with percentages of species of Paniceae of 22.6 and 22.8 respectively (Tackholm and Drar 1950). The relatively high values here undoubtedly reflect the influence of frequent flooding, which in effect introduces an "imported climate". Conversely, abnormally low values (17.4 and 16.4 per cent.) are shown for the Cuzco and Puno provinces of Peru, both predominantly at very high altitudes (Macbride 1936). A similar altitudinal effect, although on a smaller scale, is shown in the low values (17.9 and 7.0 per cent.) for the northern and southern tablelands of New South Wales (Costin 1954; Gray, personal communication). Subdivision of some of the larger regional floras would undoubtedly reveal other local anomalies, but these are too small to be shown on a world map.

Subject to the qualifications mentioned above, the most conspicuous features shown by the distribution map are as follows:

- (1) The predominantly tropical and subtropical distribution.
- (2) The zone of maximum relative concentration in north-eastern South America.
- (3) The generally higher percentages in the western hemisphere than in the eastern hemisphere.
- (4) The complexities of the distribution pattern in certain regions, notably:
 - (a) south-eastern Africa; (b) North and South America; (c) eastern Australia.

In both hemispheres, the highest percentages of species of Paniceae occur in the equatorial zone, with high values also in most other parts of the tropics. Correspondingly, all regions in latitudes above 40°, except the north-eastern United States, have less than 10 per cent. of species of the tribe. With the possible exception of this American region, there is no indication that the tribe is relatively more abundant to the north or south of the equator. On the other hand, the percentage lines do not follow closely the parallels of latitude, and even within the equatorial zone some relatively low percentages are recorded, e.g. Ecuador 35.4 per cent. (Hitchcock 1927) and Ceylon 32.1 per cent. (Senaratna 1956). Thus other factors than latitude or the biological controls caused almost solely by latitude, such as length of day, appear to affect the relative specific differentiation. Of these, high temperature appears to be the most important.

The highest recorded percentages, all above 60 per cent., occur in the Guianas and near Pernambuco in Brazil, the latter being based on data which may be somewhat unreliable. These values are as follows:

| Region | GRASS SPECIES | | | Reference |
|--------------------|-----------------|-----------------------|---------------------------|--------------------------|
| | Total Number | Number of Paniceae | Percentage of Paniceae | |
| British Guiana | 169 | 102 | 60.3 | Hitchcock 1922 |
| Suriname | 175 | 106 | 60.7 | Amshoff and Henrard 1948 |
| French Guiana | 164 | 101 | 61.6 | Lemée 1955 |
| Tapera, Pernambuco | 108 | 73 | 67.6 | Pickel 1937 |

The high values all lie within a geographically well-defined region in north-eastern South America. However, the paucity of reliable data on grass distribution in South America leaves the possibility that the region of high concentration may be much larger than is shown on the map. There are some indications that it may include the whole of the Amazon basin, for a floristic list from the very inadequately known province of Loreto, at the western edge of the Amazon basin in Peru, shows 60.7 per cent. of species of Paniceae in the grass flora (Macbride 1936).

The greater relative abundance of the Paniceae in the western hemisphere than in the eastern hemisphere is shown not only by the occurrence in South America of the zone of maximum concentration discussed above, but also by the existence of a much larger region, including the West Indies and much of central America, throughout which species of Paniceae comprise more than half the grasses. No correspondingly high values have been noted in any of the floras studied from the eastern hemisphere. Further, the relatively high percentages of Paniceae in the north-eastern United States are not paralleled in corresponding latitudes in the eastern hemisphere. Taken in conjunction, these facts suggest that the Paniceae may have originated in the western hemisphere and spread more recently to other parts of the world. This possibility, together with the significance of the complex distribution pattern in certain regions, is discussed in later sections of this paper.

IV. TAXONOMY AND THE DISTRIBUTION PATTERN

In discussing the Andropogoneae (Hartley 1958), it was shown that the indications of an Old World origin of the tribe, based on the distribution pattern, are supported by taxonomic data, since only one genus is endemic in the western hemisphere, and the more primitive genera are confined to the eastern tropics. In a similar way, the possible New World origin of the Paniceae, suggested by the distribution map, may be considered in the light of evidence from generic distribution.

It is noteworthy that none of the eight tribes into which the Paniceae *sensu lat.* have been divided by recent authors is confined to the western hemisphere, while on the other hand three or four of them (the Boivinelleae, Lecomtelleae, Trachyeae, and possibly the Melinideae) occur only in the eastern hemisphere. Further, of the 81 genera which are included within the narrower limits of the Paniceae by Pilger (1940) and Potztl (1956), 41 are found only in the eastern hemisphere. Seventeen genera occur in both hemispheres, while only 23, including the genus *Dissochondrus* which is confined to the Hawaiian Islands, are endemic to the western hemisphere. The endemic American genera do not include any which could be regarded as morphologically primitive forms.

Thus the taxonomic evidence does not support a New World origin for the tribe. Its high relative specific differentiation in the western hemisphere reflects the occurrence there of numerous species of two or three large genera which are much less abundant in the eastern hemisphere. Among these, the genus *Paspalum* is of outstanding importance. Seven of the eight subgenera of *Paspalum* recognized by Pilger (1940) are confined to the western hemisphere, while numerous species occur

in the parts of north-eastern South America where the tribe as a whole reaches its highest concentration. While, therefore, the American continent may have been a major centre of evolutionary development for certain genera, including *Paspalum*, *Axonopus*, and some sections of *Panicum*, there is no evidence that it has had a similar importance in the evolution of the tribe as a whole.

The fact that most of the smaller tribes which have been separated from Paniceae *sensu lat.* are well represented in tropical and southern Africa, including particularly Madagascar, suggests that their inclusion with the Paniceae in the distribution map may account in part for the apparently anomalous distribution pattern in south-eastern Africa. This supposition is not, however, supported by closer examination of the data. In nearly all the African floras, the number of species of the tribes allied to Paniceae *sensu str.* is small, and only in a few instances do they constitute more than 4 per cent. of the total number of grasses. Thus their omission would not greatly change the distribution pattern shown in Figure 1. In fact, insofar as they are relatively more important in the flora of south-west Africa than in the floras of regions lying to the east, their omission would tend to emphasize the apparent reversal of the distribution pattern in this latitudinal zone.

It appears, therefore, that neither the high relative specific differentiation of the Paniceae in the western hemisphere nor the complexities of the distribution pattern can be fully explained by closer study of the taxonomy of the tribe.

V. CLIMATIC FACTORS AND DISTRIBUTION

In a preliminary study of the distribution of the tribes of the grasses (Hartley 1950), it was concluded that winter temperature, while important, did not appear to be the sole factor determining the distribution of the Paniceae. It was noted that all the floras with percentages of Paniceae higher than the values expected from the temperature relationships occurred in regions of relatively high annual rainfall (> 30 in.). Conversely, all the floras with percentages below the "expected" values were in regions with mean annual rainfall below 30 inches. It was concluded: "Thus rainfall appears to be of major importance in the distribution of the tribe, but it is not possible from the data to determine the extent to which this may be responsible for the abundance of the Paniceae in the western hemisphere." With the more complete data now available, it is possible to investigate more fully the relationship between distribution, temperature, and rainfall.

The United States is a particularly convenient region for the investigation of this relationship. Fairly complete lists of grasses occurring in each of the States may be compiled from the "Manual of the Grasses of the United States" (Hitchcock 1935). This publication gives maps showing the distribution by States of about 60 per cent. of the grasses, while the distribution of the remainder may be ascertained fairly accurately from the data given. In the second edition of this manual, about 300 additional grasses are listed for the United States, but inclusion of these does not generally change the tribal percentages. The general level of reliability of the

TABLE 2
GRASS DISTRIBUTION AND CLIMATIC FACTORS IN THE UNITED STATES

| State or Region | Grass Species | | | January Tem- perature* (°F) | Annual Rainfall† (in.) |
|-------------------------------|----------------|------------------|----------------|--------------------------------------|------------------------------|
| | Total (No.) | Panicææ (No.) | Panicææ (%) | | |
| Alabama | 284 | 135 | 47.7 | 45.5 | 53.0 |
| Arizona | 304 | 36 | 11.8 | 44.2 | 13.5 |
| Arkansas | 182 | 67 | 36.8 | 42.4 | 48.1 |
| California | 361 | 28 | 7.8 | 46.1 | 24.0 |
| Colorado | 244 | 16 | 6.6 | 25.6 | 16.5 |
| Florida | 351 | 172 | 49.2 | 60.1 | 52.8 |
| Georgia | 274 | 127 | 46.3 | 47.2 | 50.2 |
| Idaho | 196 | 14 | 7.2 | 25.7 | 17.9 |
| Illinois | 206 | 55 | 26.7 | 26.7 | 36.6 |
| Indiana | 210 | 67 | 32.0 | 28.8 | 39.4 |
| Iowa | 160 | 33 | 20.6 | 18.8 | 30.9 |
| Kansas | 184 | 38 | 20.6 | 29.1 | 26.6 |
| Kentucky | 153 | 47 | 30.7 | 35.5 | 45.4 |
| Louisiana | 276 | 122 | 44.5 | 50.2 | 55.2 |
| Maine | 174 | 30 | 17.3 | 16.6 | 39.2 |
| Maryland and Delaware | 263 | 88 | 33.5 | 33.6 | 42.4 |
| Michigan | 195 | 47 | 24.1 | 19.1 | 30.4 |
| Minnesota | 146 | 30 | 20.5 | 8.3 | 25.0 |
| Mississippi | 268 | 124 | 46.4 | 47.7 | 52.9 |
| Missouri | 212 | 68 | 32.2 | 29.5 | 40.1 |
| Montana | 195 | 11 | 5.6 | 17.3 | 14.9 |
| Nebraska | 149 | 20 | 13.4 | 22.7 | 22.3 |
| Nevada | 183 | 10 | 5.5 | 29.9 | 8.8 |
| New England (excluding Maine) | 264 | 73 | 27.7 | 22.5 | 43.4 |
| New Jersey | 256 | 94 | 36.8 | 32.8 | 45.9 |
| New Mexico | 290 | 36 | 12.4 | 34.7 | 14.4 |
| New York | 215 | 52 | 24.2 | 23.5 | 39.4 |
| North Carolina | 289 | 129 | 44.6 | 42.0 | 49.8 |
| North Dakota | 119 | 12 | 10.1 | 5.4 | 16.9 |
| Ohio | 182 | 48 | 26.4 | 28.6 | 37.9 |
| Oklahoma | 202 | 59 | 29.3 | 38.0 | 31.9 |
| Oregon | 302 | 19 | 6.3 | 32.9 | 26.9 |
| Pennsylvania | 227 | 68 | 30.1 | 28.7 | 42.2 |
| South Carolina | 234 | 110 | 47.1 | 45.8 | 47.7 |
| South Dakota | 156 | 16 | 10.2 | 15.9 | 19.0 |
| Tennessee | 180 | 69 | 38.4 | 39.0 | 49.9 |
| Texas: | | | | | |
| (1) Timber belt | 238 | 97 | 40.7 | 55.4 | 46.1 |
| (2) Coastal prairies | 236 | 112 | 47.4 | 55.1 | 44.1 |
| (3) Rio Grande plains | 198 | 79 | 39.9 | 56.8 | 24.8 |
| (4) Blackland prairies | 289 | 107 | 37.0 | 48.0 | 34.5 |
| (5) Edwards Plateau | 160 | 45 | 28.1 | 48.3 | 25.5 |
| (6) Trans-Pecos area | 197 | 35 | 17.8 | 45.7 | 12.0 |
| (7) Plains country | 139 | 27 | 19.4 | 40.5 | 21.2 |

TABLE 2 (Continued)

| State or Region | Grass Species | | | January Tem- perature* (°F) | Annual Rainfall† (in.) |
|--|----------------|---------------------|-------------------|--------------------------------------|------------------------------|
| | Total (No.) | Panicaceae (No.) | Panicaceae (%) | | |
| Utah | 207 | 15 | 7.3 | 25.4 | 12.6 |
| Virginia | 242 | 95 | 39.3 | 37.7 | 37.1 |
| Washington | 263 | 13 | 5.0 | 32.9 | 35.2 |
| West Virginia | 135 | 37 | 27.4 | 32.4 | 43.4 |
| Wisconsin | 157 | 35 | 22.3 | 13.9 | 30.6 |
| Wyoming | 189 | 9 | 4.8 | 21.5 | 13.9 |
| San Diego Co., Cal. | 160 | 23 | 14.4 | 49.1 | 24.7 (1)‡ |
| Douglas Co., Kan. | 111 | 21 | 18.9 | 29.8 | 35.5 (2) |
| Cedar Co., Iowa | 87 | 20 | 23.0 | 21.4 | 33.7 (3) |
| Clinton Co., Ohio | 86 | 27 | 31.4 | 30.7 | 46.5 (4) |
| Washington, D.C. | 78 | 27 | 34.6 | 33.7 | 40.1 (5) |
| South-western Georgia | 195 | 84 | 43.1 | 54.3 | 50.6 (6) |
| Cayuga Quadrangle, N.Y. | 153 | 34 | 22.2 | 25.4 | 35.0 (7) |
| Tidewater-Piedmont region (Va. and Md.) | 231 | 86 | 37.2 | 33.7 | 42.1 (8) |
| Jornada Exp. Range, New Mexico | 84 | 16 | 19.1 | 38.3 | 10.3 (9) |

* Arithmetic means of reporting stations cited by United States Department of Agriculture (1936).

† State unit values as given by United States Department of Agriculture (1941), and arithmetic means of reporting stations in smaller areas.

‡ References: (1) Higgins 1949. (2) McGregor 1948. (3) Fay 1951. (4) Terrell 1955. (5) Freeman 1953. (6) Thorn 1954. (7) Clausen 1949. (8) Gilman 1957. (9) Little and Campbell 1943.

data may be judged by comparing the percentages derived from Hitchcock's Manual with those calculated from independent floras for certain States:

| State | Panicaceae (% of species) | | Reference |
|--------------|---------------------------|----------------|-------------------------------------|
| | Hitchcock 1935 | State Flora | |
| Arizona | 11.8 | 12.7 | Kearney and Peebles 1951 |
| California | 7.8 | 8.2 | Sampson, Chase, and Hedrick 1951 |
| Illinois | 26.7 | 25.6 | Jones 1945 |
| Kansas | 20.6 | 20.5 | Gates 1940 |
| Pennsylvania | 30.1 | 26.3 | Pohl 1947 |

The figures show a good level of agreement for each of the States concerned, with the partial exception of Pennsylvania. The occurrence of grass species in this State cannot be determined with accuracy from the small-scale distribution maps given in Hitchcock's Manual, while the flora includes a relatively large proportion of waifs which are doubtfully naturalized. The percentage of species of Panicaceae in

the various States covers a wide range, from less than 5 per cent. for Wyoming to nearly 50 per cent. for Florida.

Reliable temperature and rainfall data are available for numerous reporting stations in each of the States, together with State rainfall averages (United States Department of Agriculture 1936, 1941). It is thus possible to compare the grass percentages with the mean January temperature and mean annual rainfall for corresponding areas throughout the United States. Similar data are not readily available for any region of comparable size elsewhere in the world.

The relevant floristic data, as well as mean January temperature and mean annual rainfall for each of the American States, are shown in Table 2. Because

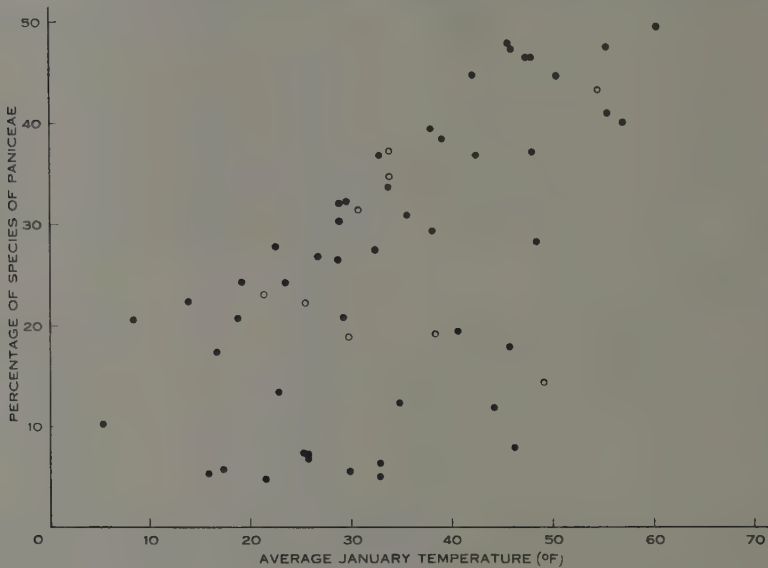


Fig. 2.—Distribution of Paniceae in relation to midwinter temperature in the United States. ● Data based on flora of State from Hitchcock's Manual. ○ Data based on other regional flora.

of their small size, Maryland and Delaware are grouped together, as are the New England States with the exception of Maine. On the other hand, Texas has been subdivided into the seven phytogeographical regions recognized by Cory and Parks (1937). Data for nine other smaller or overlapping regions in various parts of the United States are also given at the end of Table 2.

The relationships between grass distribution and January temperatures and between grass distribution and annual rainfall are shown graphically in Figures 2 and 3. While the interpretation of these figures is impeded by the existence of a general relationship between high winter temperatures and high annual rainfall in the United States, certain tentative conclusions may be drawn from them.

There is an indication in Figure 2 that the level of January temperature is an important factor limiting the percentage of species of Paniceae in the grass flora. For each winter temperature range, there is an upper limit to the percentage of

species of *Panicum*, as shown by the linear relationship between maximum percentage and temperature. On the other hand, percentages much below the maximum occur at each temperature level, which indicates that some factors other than winter temperature are also of importance in relation to distribution.

The relationship between grass frequency and annual rainfall shown in Figure 3 is a most striking one, suggesting strongly that rainfall is at least one of the other major factors concerned. There is a very high positive correlation ($r = +0.86$) between the percentage of species of *Panicum* in the grass flora of the several States

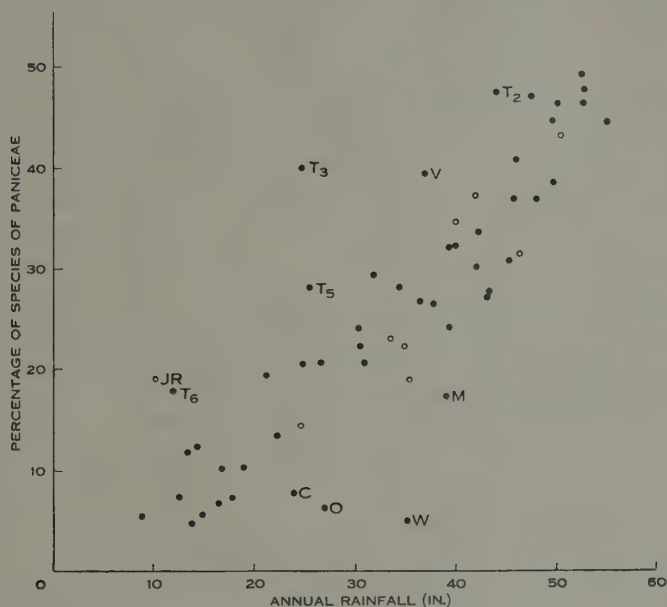


Fig. 3.—Distribution of *Panicum* in relation to annual rainfall in the United States. ● Data based on flora of State from Hitchcock's Manual. ○ Data based on other regional flora. JR, Jornada Experimental Range, New Mexico. C, California. M, Maine. O, Oregon. V, Virginia. W, Washington. T₂, Coastal prairies, Texas. T₃, Rio Grande plains, Texas. T₅, Edwards Plateau, Texas. T₆, Trans-Pecos area, Texas.

and the mean annual rainfall of those States. Further, the points based on the data for the smaller areas, shown by open circles in the diagram, fit well into the general pattern. The only noteworthy exception is the relatively high percentage shown for the Jornada Experimental Range in New Mexico, but as this is a small area with great variations in altitude the rainfall figure may not be fully representative.

The points based on data from those States which deviate somewhat from the general pattern are indicated in Figure 3. Thus, Washington (W), Oregon (O), California (C), and Maine (M) have relatively low percentages, while most parts of Texas (T₂, T₃, T₅, T₆) and Virginia (V) have relatively high values. Most of these deviations appear to be associated with the seasonal distribution of the rainfall. In Texas the rain falls predominantly during the summer months when plants are

actively growing, while in the western States it falls mainly in winter, when, especially in the northern parts, temperatures are too low for active plant growth. It appears probable, therefore, that high percentages of Paniceae are associated with a long growing season, during which both temperature and rainfall are adequate for active growth, rather than with high annual rainfall as such.

Examination of the world distribution map (Fig. 1) reveals that the percentages of species of Paniceae show a similar association with high winter temperature and high rainfall in other regions. This is evident not only in the zones of high concentration in both the western and eastern hemispheres, but also in those zones with a complex distribution pattern which have been mentioned above. Thus in southern Africa, an increase in the percentage of Paniceae from west to east is matched by a corresponding increase in mean annual rainfall, which varies from less than 10 inches for most of south-west Africa to more than 30 inches for Mozambique. The effect of the length of growing season is evident in Australia, where the highest percentages of Paniceae are shown in south-eastern Queensland. As shown by Miles (1947), this region has a longer growing season than regions of greater annual rainfall further north.

It is concluded that there is a very close relationship between the distribution of the Paniceae, based on percentage frequency, and climatic factors. Winter temperature and annual rainfall are of special importance, partly through their effect on the length of the growing season.

VI. GENERAL DISCUSSION

It has been shown in this paper that the pattern of geographical distribution of the tribe Paniceae is a well-defined and coherent one, showing an apparent relationship to present climatic factors. This suggests strongly that the tribe is a natural, monophyletic one. This conclusion is in accordance with the views of most taxonomists, as well as with the evidence from histological, anatomical, embryological, and cytological studies (Avdulov 1931; Prat 1936; Reeder 1957; Tateoka 1957). Because of the relatively small numbers of species involved, it is not possible to draw conclusions from the distribution pattern about the relationship between the Paniceae and the smaller tribes which have been separated from it by modern authorities. These smaller tribes have been separated and defined mainly on morphological grounds (cf. Potztal 1956), and little information is available about their cytology and anatomy.

The greater relative abundance of the Paniceae in the western hemisphere is *prima facie* evidence that the tribe may have originated in that region, but, as indicated above, this conclusion is not supported by closer investigation of the taxonomy. The close relationship shown to exist between climatic factors and distribution, suggests that the higher relative specific differentiation in the western hemisphere may in part reflect the more general occurrence there of climates favourable to the development of the tribe. Further, the concentration of the other great tribe of tropical grasses, the Andropogoneae, in the eastern hemisphere (Hartley 1958) would in itself tend to produce higher percentages of species of Paniceae in the western hemisphere.

It is of some interest to consider the relationship between the tribes Paniceae and Andropogoneae in the light of the geographical evidence. Together with the small tribe Maydeae and the other small tribes separated from the Paniceae *sensu lat.*, these constitute the subfamily Panicoideae as recognized by most taxonomists.* This subfamily is again a very natural one, as indicated both by taxonomic and other evidence.

The geographical evidence, taken in conjunction with other taxonomic data discussed above, suggests that the Panicoideae most probably originated in the warmer regions of the eastern hemisphere, and perhaps in the East Africa-Madagascar area. It is noteworthy that two of the tribes which have been separated from the Paniceae *sensu lat.*, the Lecomtelleae and Boivinelleae, are endemic in Madagascar, and have morphological characteristics which are in some respects intermediate between those of the Andropogoneae and the Paniceae. Further, the occurrence in southern Africa of *Miscanthidium*, a relatively primitive genus of Andropogoneae, is consistent with the possible origin of the Panicoideae in this region.

From an origin in East Africa or elsewhere in the warmer parts of the eastern hemisphere, the Panicoideae spread through the tropical and subtropical parts of both hemispheres. It appears that the tribe Paniceae formed the advance guard of this spread, reaching the western hemisphere at an early stage, and developing rapidly there under favourable climatic conditions. The Andropogoneae, possibly formed at a later stage from the panicoid stock, spread rather towards the Indo-Malaysian region, and only reached the American continent comparatively recently.

It is of special interest to note that the relative development of the two tribes is associated with, and possibly determined by, differences in their climatic adaptation. Both tribes reach their highest development in hot, moist climates. However, while the Paniceae are especially characteristic of regions with a long wet season, such as are found in the equatorial belt, the Andropogoneae reach their highest relative development in monsoonal climates, with a short season of heavy rainfall. This is shown by their general distribution throughout the world, and may be illustrated by comparison of the monthly rainfall of Khandala, in the Western Ghats of India, which has the highest recorded percentage of Andropogoneae, with that of Georgetown, in British Guiana, in the zone of highest concentration of Paniceae. Both regions have a high annual rainfall (Khandala 216 in., Georgetown 90 in.), but while the rainfall of Georgetown is fairly well distributed through the year, that of Khandala has a very pronounced seasonal peak.

| | Percentage of Annual Rainfall in Each Month† | | | | | | | | | | | |
|------------|--|------|------|------|------|------|------|------|-------|------|------|------|
| | Jan. | Feb. | Mar. | Apr. | May | June | July | Aug. | Sept. | Oct. | Nov. | Dec. |
| Khandala | 0.0 | 0.0 | 0.0 | 0.0 | <1.0 | 13.4 | 40.8 | 33.5 | 10.9 | 1.1 | 0.0 | 0.0 |
| Georgetown | 9.4 | 6.6 | 7.5 | 7.0 | 12.4 | 13.1 | 11.1 | 7.2 | 3.4 | 2.8 | 6.5 | 13.0 |

* Some recent authors, e.g. Brown, Heimsch, and Emery (1947), follow Prat (1936) in including within the Panicoideae the Chloridoideae and some smaller taxa. The geographical distribution of these groups will be discussed in a subsequent paper.

† Data from Santapau (1953) and United States Department of Agriculture (1941).

The differing climatic responses are in turn determined by different patterns of physiological development, and it appears that these patterns are no less characteristic of the tribes than are the morphological features which form the basis of taxonomic classifications.

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VIII. REFERENCES

- AMSHOFF, G. J. H., and HENRARD, J. TH. (1948).—Gramineae. In A. Pulle, "Flora of Suriname".
- AVDULOV, N. P. (1931).—Karyo-systematische Untersuchung der Familie Gramineen. *Bull. Appl. Bot. Pl.-Breed. Suppl.* **43**: 428.
- BENTHAM, G., and HOOKER, J. D. (1883).—"Genera Plantarum." Vol. 3. (L. Reeve & Co.: London.)
- BEWS, J. W. (1929).—"The World's Grasses." (Longmans, Green & Co.: London.)
- BROWN, W. V., HEIMSCH, C., and EMERY, W. H. P. (1957).—The organization of the grass shoot apex and systematics. *Amer. J. Bot.* **44**: 590-5.
- CLAUSEN, R. T. (1949).—Checklist of the vascular plants of the Cayuga Quadrangle, 42-43°N, 76-77°W. Mem. Cornell Agric. Exp. Sta. No. 291.
- CORY, V. L., and PARKS, H. B. (1937).—Catalogue of the flora of the State of Texas. *Bull. Tex. Agric. Exp. Sta.* No. 550.
- COSTIN, A. B. (1954).—"A Study of the Ecosystems of the Monaro Region of New South Wales." (Govt. Printer: Sydney.)
- FAY, M. J. (1951).—Flora of Cedar County, Iowa. *Proc. Iowa Acad. Sci.* **58**: 107-31.
- FREEMAN, O. M. (1953).—Annotated list of the plants growing naturally at the National Arboretum. *Contr. (U.S.) Nat. Arbor.* No. 1.
- GATES, F. C. (1940).—Annotated list of the plants of Kansas: ferns and flowering plants. *Contr. Bot. Dep. Kans. St. Coll.* No. 391.
- GILMAN, E. M. (1957).—Grasses of the Tidewater-Piedmont region of northern Virginia and Maryland. *Castanea* **22**: 1-105.
- HACKEL, E. (1887).—Gramineae. In A. Engler and K. Prantl, "Die Naturlichen Pflanzenfamilien". Vol. 2. Part 2.
- HARTLEY, W. (1950).—The global distribution of tribes of the Gramineae in relation to historical and environmental factors. *Aust. J. Agric. Res.* **1**: 355-73.
- HARTLEY, W. (1958).—Studies on the origin, evolution, and distribution of the Gramineae. I. The tribe Andropogoneae. *Aust. J. Bot.* **6**: 115-28.
- HIGGINS, E. B. (1949).—Annotated distributional list of the ferns and flowering plants of San Diego County, California. *Occ. Pap. S. Diego Soc. Nat. Hist.* No. 8.
- HITCHCOCK, A. S. (1922).—Grasses of British Guiana. *Contr. U.S. Nat. Herb.* **22**(6): 439-515.
- HITCHCOCK, A. S. (1927).—Grasses of Ecuador, Peru, and Bolivia. *Contr. U.S. Nat. Herb.* **24**(8): 291-556.
- HITCHCOCK, A. S. (1935).—"Manual of the Grasses of the United States." (U.S. Govt. Print. Off.: Washington.)
- HUBBARD, C. E. (1934).—Gramineae. In J. Hutchinson, "The Families of Flowering Plants. II. Monocotyledons". (Macmillan & Co. Ltd.: London.)
- JONES, G. N. (1945).—Flora of Illinois. *Amer. Midl. Nat. Monogr.* No. 2.
- KEARNEY, T. H., and PEEBLES, R. H. (1951).—"Arizona Flora." (Univ. of California Press: Berkeley.)

- KUNTH, C. S. (1833).—"Enumeratio Plantarum. Tomus Primus et Suppl." (J. G. Cottae: Stuttgart.)
- LEMÉE, A. (1955).—"Flora de la Guyana française." Vol. 1. (Paul Lechevalier: Paris.)
- LITTLE, E. L., and CAMPBELL, R. S. (1943).—Flora of Jornada Experimental Range, New Mexico. *Amer. Midl. Nat.* **30**: 626-70.
- MACBRIDE, J. F. (1936).—Flora of Peru: Part 1. Publ. Field Mus. No. 351.
- MCGREGOR, R. L. (1948).—The flora of Douglas County, Kansas. *Trans. Kans. Acad. Sci.* **51**: 77-106.
- MILES, J. F. (1947).—The pastoral and agricultural growing season in north-eastern Australia. *J. Aust. Inst. Agric. Sci.* **13**: 41-9.
- PICKEL, D. B. (1937).—Catalogo do herbario da Escola Superior de Agricultura em Tapera. *Bol. Mus. Nac. Rio de J.* **13**: (1-2).
- PILGER, R. (1940).—Gramineae III (Unterfam. Panicoideae). In A. Engler and K. Prantl, "Die Naturlichen Pflanzenfamilien". Vol. 14e. (W. Engelmann: Leipzig.)
- PILGER, R. (1954).—Das System der Gramineae. *Bot. Jb.* **76**: 281-384.
- POHL, R. W. (1947).—A taxonomic study on the grasses of Pennsylvania. *Amer. Midl. Nat.* **38**: 513-604.
- POTZTAL, E. (1956).—Nachtrag zu Gramineae III. In A. Engler and K. Prantl, "Die Naturlichen Pflanzenfamilien". Vol. 14d. (Duncker & Humblot: Berlin.)
- PRAT, H. (1936).—La systématique des Graminées. *Ann. Sci. Nat. (Bot.)* (10)**18**: 165-258.
- REEDER, J. R. (1957).—The embryo in grass systematics. *Amer. J. Bot.* **44**: 756-68.
- SAMPSON, A. W., CHASE, A., and HEDRICK, D. W. (1951).—California grasslands and range forage grasses. Bull. Calif. Agric. Exp. Sta. No. 724.
- SANTAPAU, H. (1953).—Flora of Khandala on the Western Ghats of India. *Rec. Bot. Surv. India* **61**(1): 1-396.
- SENARATNA, S. D. J. E. (1956).—"The Grasses of Ceylon." (Govt. Printer: Peradeniya.)
- STEUDEL, E. G. (1855).—"Synopsis Plantarum Glumacearum." (Stuttgart.)
- TACKHOLM, V., and DRAR, M. (1950).—Flora of Egypt. Vol. 1. Fac. Sci. Fouad I Univ. Bull. No. 17.
- TATEOKA, T. (1957).—Miscellaneous papers on the phylogeny of Poaceae (10). Proposition of a new phylogenetic system of Poaceae. *J. Jap. Bot.* **32**: 275-87.
- TERRELL, E. E. (1955).—The vascular flora of Clinton County, Ohio. *Ohio J. Sci.* **55**: 215-40.
- THORN, R. F. (1954).—The vascular plants of southwestern Georgia. *Amer. Midl. Nat.* **52**: 257-327.
- UNITED STATES DEPARTMENT OF AGRICULTURE (1936).—"Atlas of American Agriculture." (U.S. Govt. Print. Off.: Washington.)
- UNITED STATES DEPARTMENT OF AGRICULTURE (1941).—"Climate and Man." 1941 Yearb. Agric. (U.S. Govt. Print. Off.: Washington.)

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